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Effect of dietary supplementation of selenium nanoparticles on growth performance and nutrient digestibility of common carp (*Cyprinus carpio* Linnaeus, 1758) fingerlings fed sunflower meal based diet

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ABSTRACT

Sunflower meal based diet supplemented with selenium nanoparticles (Se NPs) at levels of 0, 1, 2, 3, 4 and 5 mg kg^{-1} were fed to common carp (*Cyprinus carpio* Linnaeus, 1758) fingerlings in a seventy days feeding trial, to examine the effects on growth, nutrient digestibility and heamatology. Triplicate tanks were used and each tank contained 15 fingerlings that were subjected to experimental feeding at 4% of their live wet weight. A non-digestible marker, chromic oxide was added in the feed at 1% level. Growth performance in terms of weight gain %, feed conversion ratio (FCR) and specific growth rate (SGR) as well as nutrient digestibility (crude protein, crude fat, gross energy) were estimated following standard methods/standard formulae. Maximum weight gain% (210%), SGR (1.26) and best FCR (1.26) as well as highest nutrient digestibility (crude protein 71%, crude fat 72% and gross energy 70%) were observed in fish fed test diet III supplemented with 2 mg kg^{-1} Se NPs. These results showed that Se NPs supplementation at the rate of 2 mg kg^{-1} level is beneficial for improvement of growth in *C. carpio* fingerlings.

Keywords: *Cyprinus carpio*, Growth, Nutrient digestibility, Selenium nanoparticles

Introduction

The common carp (*Cyprinus carpio* Linnaeus, 1758), is the most common teleost species which is grown as an important source of food in about one hundred countries throughout the world, with its production outrunning 3.79 million t in 2012 (Lv *et al*., 2016). It is being cultured in Eurasian countries, for thousands of years (Zhang *et al*., 2013). In contrast to shrimps and salmons, carps are considered as ecofriendly fish because they are omnivorous in their feeding habit (Xu *et al*., 2014).

Due to increasing demand for food world over, quality nutrition is a major problem to be focused, particularly in underdeveloped countries (Abdulkadir *et al*., 2016). Aquaculture is considered as one of the best alternative protein source and is now a growing industry owing to the need for good quality protein (Rowland *et al*., 1991). Fish culture is of particular importance because they contribute to cheap as well as best source of protein for human consumption (Javed and Usmani, 2013), as it contains high quality nutrients such as amino acids, vitamins, minerals as well as fatty acids (Zhou *et al*., 2004; Dawood *et al.,* 2015). Intensification of aquaculture practices led to the need for development of quality feeds (Sanchez-Hernandez *et al*., 2016). Though it is a profitable industry, still much is to be done to make aquaculture a practicable industry, and the challenge is to produce environment friendly feed for fish (Hussain *et al*., 2015).

The success of aquaculture is dependent on the quality of feed used. So, formulation of nutritionally balanced feed for fish should be a major point to be focused (Winkaler *et al.,* 2007). A carefully formulated and well prepared feed for fish plays an important role in fish culture. However, unbalanced supply, rising demand and high cost of fish meal (FM) made it crucial to look for alternate substitutes (Rowland *et al*., 1991). To support aquaculture development, it is necessary to identify unconventional protein sources (Baruah *et al*., 2004; Lunger *et al.,* 2007). Utilisation of other improved protein sources and reduction in fish meal consumption was recommended by many researchers (Barnes *et al*., 2012; Dedeke *et al.,* 2013). Plant byproducts are considered as best replacement of fish meal in fish diet by many researchers owing to comparatively lower cost and easy availability throughout the year (Hussain *et al*., 2015; Wang *et al*., 2015). Beneficial effects of plant meal on fish growth were also investigated (Hussain *et al.,* 2011).

The efficiency of different protein sources as the complete or partial replacement of FM has been evaluated in fish feed (El-Saidy and Gaber, 2002). Sunflower meal is considered as a good dietary protein source in animal diet (Olivera-Castillo and Martinez-Palacios, 2002). Sunflower crop which can be cultivated two to three times in a year in tropical areas, is an important source of nutrients and is relatively inexpensive source of protein (Lu *et al*., 2013). Sunflower meal (SFM) has been extensively used as a protein source for animals. It consists of 45-48% crude protein content after oil extraction (Mushtaq *et al.,* 2006). The development of nanotechnology has brought new applications to many fields (Lin *et al.,* 2004). Nanomaterials possess one or more external dimensions on the scale from 1 to 100 nm and these materials have unique properties due to the high surface to volume ratio (Abdel-Tawwab *et al*., 2007). Selenium (Se) is considered as an important trace element for fish and has been considered as an essential nutrient for aquaculture (Ashouri *et al*., 2015). Dietary Se administration has been observed to improve the growth performance in various fish species (Gatlin and Wilson, 1984; Jaramillo and Gatlin, 2004; Zhou *et al*., 2009). The present research work was performed to evaluate the effect of selenium nanoparticles (Se NPs) supplemented SFM based diet on growth performance of *C. carpio.*

Materials and methods

Fish and experimental conditions

C. carpio fingerlings were brought from a local fish seed hatchery in Faisalabad and were acclimatised to the laboratory conditions for two weeks. Fingerlings were stocked in specially designed V-shaped water tanks (having water holding capacity of 70 l). During the period of acclimatisation the fingerlings were fed on the basal diet once daily (Rowland *et al*., 1991). Water quality parameters such as pH, temperature and dissolved oxygen were monitored regularly and maintained at optimum levels. The supply of oxygen through capillary system was also maintained using the air pump. Before start of the experiment, fingerlings of *C. carpio* were given dip treatment with 0.5% saline solution (Rowland *et al*., 1991).

Experimental design

Experimental diet was parted into one control and five test diets and supplemented with graded levels (0, 1, 2, 3, 4 and 5 mg kg^{-1}) of Se NPs. For each treatment, triplicate tanks were used. Fifteen fingerlings were stocked in each replicate. The fingerlings were subjected to feeding at 4% of their live wet weight for a period of 70 days. SFM based diets supplemented with Se NPs were tested along with the control diet to determine the growth and nutrient digestibility following completely randomised design (CRD).

Processing of Se NPs

Stock solution of Se NPs (SIGMA ALDRICH, 0.5 mg of Se salt in 10 ml of ultrapure water) was sonicated for 8 h, and then 1, 2, 3, 4 and 5 ml each of the stock solution was added to 49, 48, 47, 46 and 45 ml of distilled water respectively. The diluted Se NPs were subjected to sonication for further 15 min to assure even delivery of material in the experimental diets. To incorporate the diluted Se NP solutions in the respective diets, the methods described by Coyle *et al.* (2004) and Handy *et al*. (2005) were followed.

Preparation of pellet feed

The ingredients of the experimental feed procured from a local feed mill, were finely ground and passed through a 0.3 mm sieve. Analysis of chemical composition of the feed ingredients was done following standard methods (AOAC, 1995), prior to formulation of experimental diets (Table 1). Chromic oxide (Cr_2O_3) was added as an inert marker in all the diets at the rate of 1%. All feed ingredients were put together in a mixer for 5 min and fish oil was added gradually thereafter. Ingredients were mixed slowly in the mixer after the addition of 10-15% of water, to make a suitably textured dough and was processed further using a pelleting machine to make floating pellets (AOAC, 1995). One control diet and five test diets were prepared using the SFM based diet by spraying graded levels (0, 1, 2, 3, 4, 5 mg kg^{-1}) of Se NPs. The required concentrations (0, 1, 2, 3, 4, and 5 mg kg^{-1}) of Se NPs were prepared in 20 ml of distilled water and sprayed on one kg of each test diet. Same amount of water was also sprayed on the control diet (without Se NP supplementation) to keep the amount of moisture equivalent. All the diets were then dried in a shady place and stored at 4°C until use.

Feeding protocol and sample collection

Fingerlings of *C. carpio* were fed at 4% of live wet weight on their prescribed diet twice daily. After 2 h of feeding session, the left over diet was siphoned out from each tank. After 2 h of feeding session, faecal matter was collected carefully to avoid breakage of thin faecal strings to minimise the leaching of nutrients. Faeces were dried in oven at 65°C, homogenised and stored for further chemical analysis.

Growth study

Fingerlings with an average weight of 8.07±0.041 g $fish^{-1}$ were stocked in each replicate tanks and from each tanks, fishes were bulk weighed fortnightly during the experimental period to evaluate the growth performance. Weight gain, FCR (feed conversion ratio), SGR (specific growth rate) and weight gain% of fish fingerlings were estimated using standard formulae:

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

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

$$\text{SGR \%} = \frac{(\ln \text{final wt. of fish} - \ln \text{initial wt. of fish})}{\text{Duration of feeditng}} \times 100$$

Chemical analysis of feed and faeces

Moisture content of test diets, faeces and ingredients were calculated after oven-drying of homogenised samples at 105°C for 12 h. Micro Kjeldahl Apparatus (InKjel M behr Labor Technik GmbH D-40599 Dusseldorf) was used to determine the crude protein (CP) (N × 6.25) whereas Soxhlet system (Soxhlet Extraction Heating Mantels, 250 ml 53868601) was used to check the amount of crude fat by petroleum ether extraction (EE) method. Crude fiber (CF) contents were calculated as loss on ignition of dried lipid-free residues after digestion with 1.25% H_2SO_4 and 1.25% NaOH whereas ash was determined by ignition at 650°C for 12 h in electric furnace (Naberthern B170) to constant weight. Total carbohydrate (N-free extract) was determined using the formula: Total carbohydrate % =100 - (EE % + CP % + Ash % + CF%). Oxygen bomb calorimeter was used to estimate the gross energy.

Statistical analysis

One-way analysis of variance (ANOVA) was applied on data collected from experimental feeding to analyse growth performance as well as nutrient digestibility (Steel *et al.,* 1960). For comparing between the treatments, Tukey's honesty significant difference test was used and was considered significant at $p<0.05$ (Snedecor and Cochran, 1956). The CoStat Computer Package (version 6.303, PMB 320, Monterey, CA, 93940 USA) was used for statistical analysis.

Results and discussion

In the present study, different levels of Se NPs (0, 1, 2, 3, 4 and 5 mg kg^{-1}) were supplemented in SFM based diet to determine the effect of Se NPs supplementation on growth of *C. carpio* fingerlings. Maximum values for weight gain (16.18 g), weight gain % (211%) and SGR (1.26) were observed for the Test diet-III supplemented with 2 mg kg^{-1} of Se NPs. The second highest value of weight gain (14 g), weight gain % (176%) and SGR (1.13) was noted for 3 mg kg^{-1} of Se NPs supplementation. Values of weight gain were significantly ($p<0.05$) low (5.75 g) in fingerlings when fed on 5 mg kg^{-1} of Se NPs supplemented diet compared to that of control diet (9.4 g), while there was no significant difference in weight gain (9.16 g) in fingerlings fed on 4 mg kg^{-1} of Se NPs supplemented diet compared to control diet. Fishes fed on other test diets (1, 2 and 3 mg kg^{-1} of

Table 1. Composition (%) of control and test diets

Ingredients	Control diet	Test Diet II	Test Diet-III	Test Diet-IV	Test Diet-V	Test Diet-VI
Nano-Selenium (mg kg^{-1})	0	1	2	3	4	5
Sunflower meal (SFM)	55	55	55	55	55	55
Fish meal	14	14	14	14	14	14
Wheat flour*	12	12	12	12	12	12
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

Chemical composition (%) of feed ingredients

Ingredients	Dry matter (%)	Crude protein (%)	Crude fat (%)	Crude fiber (%)	Ash (%)	Gross energy (kcal g^{-1})	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
Wheat flour	92.41	10.08	2.37	2.66	2.06	2.98	82.83

*Nanoparticles were added at the cost of wheat flour

** Vitamin premix (per kg): Vitamin D_3: 3,000,000 IU; Vitamin A: 15,000,000 IU; Vitamin E: 30,000 IU; Vitamin B_1: 3,000 mg; Vitamin B_6: 4,000 mg; Vitamin B_{12}: 40 mg; Vitamin B_2: 7,000 mg; Vitamin C: 15,000 mg; Vitamin K_3: 8,000 mg; Folic acid: 1,500 mg; Calcium pantothenate: 12,000 mg; Nicotinic acid: 60,000 mg

*** Mineral premix (per kg): Mn: 2000 mg; Ca: 155 g, Zn: 3,000 mg; Cu : 600 mg; Co: 40 mg; I: 40 mg; P: 135 g;, Fe: 1,000 mg; Mg: 55 g; Se: 3 mg; Na: 45 g

Se NPs) recorded significantly higher ($p<0.05$) weight gain compared to control diet, with Test diet-III showing the highest weight gain (16.18 g) Maximum value for FCR (1.99) was observed at 5 mg kg^{-1} of Se NPs supplementation in SFM based diet. The second highest value (1.86) was found for 4 mg kg^{-1} of Se NPs supplemented diet followed by fish fed 0 mg kg^{-1} without Se NPs supplementation. Lowest/best FCR (1.34) was observed in the fish fed on Test diet-III with 2 mg kg^{-1} level of Se NPs (Table 2).

Nutrient composition of feed, faeces and digestibility were analysed and the results are presented in Table 3. Nutrient contents in all diets including control diet were similar with each other except for the supplementation of Se NPs. But highest level of protein (17.83%) was found to be excreted through faeces in case of fish fed Test diet-VI supplemented with 5 mg kg^{-1} Se NPs followed by control diet (16.55%). Crude fat (3.96%) and gross energy (1.84 kcal g^{-1}) in faeces were also found to be highest in case of Test diet-VI. The current results showed that minimum discharge of crude protein (9.70%) and gross energy (1.10 kcal g^{-1}) through faeces was observed when *C. carpio* fingerlings were fed at 2 mg kg^{-1} level of Se NPs based diet (Test diet-III). However, lowest amount of fat (1.32) was discharged at 1 mg kg^{-1} (Table 3). It was also obvious from the results that the maximum digestibility values for crude protein (71%), crude fat (72%) and gross energy (70 kcal g^{-1}) for *C. carpio* fingerlings were observed at 2 mg kg^{-1} Se NPs supplemented diet. In contrast, significantly ($p<0.05$) low levels of crude protein (46%), crude fat (49%) and gross energy (51% kcal g^{-1}) digestibility were recorded in fingerlings fed 5 mg kg^{-1} Se NPs supplemented diet (Table 4). On the basis of these results, it was concluded that, fish fed on SFM based diet with the supplementation of 2 mg kg^{-1} Se NPs showed higher performance as compared to fish fed with control as well as other test diets.

Results of the present study clearly indicated that Se NPs supplementation at 2 mg kg^{-1} is optimum for maximum growth performance of common carp fingerlings. *C. carpio* fingerlings fed on a diet supplemented with 2 mg kg^{-1} Se NPs recorded the best FCR value as well as maximum SGR% and weight gain% values. The present results are quite similar to the findings of Ashouri *et al.* (2015) who concluded that the supplementation of Se helped to improve growth of common carp. Han *et al.* (2011) also determined the optimum requirement of Se for gibel carp (*Carassius auratus gibelio*) as 1.18 mg nano-Se kg^{-1}. Lin and Shiau (2005) while working on Se NPs supplementation for grouper *Epinephelus malabaricus*, reported that fish exhibited increased growth at supplementation levels of 0.77 mg Se kg^{-1}. Growth of channel catfish (*Ictalurus punctatus*) was found affected by the supplementation of Se in diet (Gatlin and Wilson,

Table 2. Growth performance of *C. carpio* fingerlings fed on SFM based test diets supplemented with Se NPs

Experimental diets	Levels of Se NPs (mg kg^{-1})	Initial weight (g)	Final weight (g)	Weight gain (g)	Weight gain %	Wt. gain fish per day	Feed intake	FCR	SGR (%)
Test diet-I (control)	0	7.68±0.19^{a}	17.08±0.22^{d}	9.40±0.19^{d}	122.55±4.36^{d}	0.134±0.003^{d}	0.25±0.01b^{c}	1.85±0.04^{a}	0.89±0.02^{d}
Test diet-II	1	7.70±0.17^{a}	19.68±0.24^{c}	11.98±0.08^{c}	155.69±2.67^{c}	0.171±0.001^{c}	0.25±0.01b^{c}	1.48±0.06^{b}	1.04±0.01^{c}
Test diet-III	2	7.69±0.26^{a}	23.87±0.48^{a}	16.18±0.23^{a}	210.55±4.69^{a}	0.231±0.003^{a}	0.31±0.02^{a}	1.34±0.06^{b}	1.26±0.02^{a}
Test diet-IV	3	7.68±0.18^{a}	21.22±0.27^{b}	13.53±0.15^{b}	176.19±4.04^{b}	0.193±0.002^{b}	0.28±0.01^{ab}	1.45±0.04^{b}	1.13±0.02^{b}
Test diet-V	4	7.67±0.12^{a}	16.83±0.58^{d}	9.16±0.46^{d}	119.43±4.27^{d}	0.131±0.007^{d}	0.24±0.02^{c}	1.86±0.06^{a}	0.87±0.02^{d}
Test diet-VI	5	7.70±0.24^{a}	13.46±0.47^{e}	5.75±0.34^{e}	74.71±4.46^{e}	0.082±0.005^{e}	0.16±0.01^{d}	1.99±0.04^{a}	0.62±0.03^{e}

Means within rows having different superscripts are significantly different ($p<0.05$). Data are means of three replicates.
SGR = Specific growth rate, FCR= Feed conversion ratio

Table 3. Apparent crude protein (CP), ether extract (EE) and gross energy (GE) of feed and faeces of *C. carpio* fingerlings fed on SFM based diet supplemented with Se NPs

Experimental diets	Levels of Se NPs (mg kg^{-1})	CP (%) in diet	CP (%) in faeces	EE (%) in diet	EE (%) in faeces	GE (kcal g^{-1}) in diet	GE (kcal g^{-1}) in faeces
Test diet-I (control)	0	31.52±0.12^{a}	16.55±0.28^{d}	7.31±0.08^{a}	3.53±0.12^{b}	3.52±0.10^{a}	1.53±0.05^{b}
Test diet-II	1	31.52±0.07^{a}	14.09±0.64^{c}	7.30±0.07^{a}	3.02±0.12^{c}	3.53±0.10^{a}	1.32±0.05^{b}
Test diet-III	2	31.52±0.07^{a}	9.70±0.38^{a}	7.31±0.05^{a}	2.13±0.15^{d}	3.53±0.14^{a}	1.10±0.05^{c}
Test diet-IV	3	31.52±0.09^{a}	11.75±0.91^{b}	7.31±0.09^{a}	2.84±0.24^{c}	3.53±0.09^{a}	1.46±0.11^{b}
Test diet-V	4	31.53±0.19^{a}	14.25±0.24^{c}	7.30±0.07^{a}	3.09±0.13^{c}	3.52±0.23^{a}	1.52±0.11^{b}
Test diet-VI	5	31.51±0.15^{a}	17.83±0.23^{e}	7.32±0.08^{a}	3.96±0.09^{a}	3.52±0.10^{a}	1.84±0.08^{a}

Means within rows having different superscripts are significantly different ($p<0.05$). Data are means of three replicates.

Table 4. Apparent crude protein (CP), ether extract (EE) and gross energy (GE) levels in *C. carpio* fingerlings fed on SFM based diet supplemented with Se NPs

Experimental diets	Levels of Se NPs (mg kg^{-1})	CP (%)	EE (%)	GE (kcal g^{-1})
Diet-I (Control)	0	49.78 ± 0.92^{d}	53.82 ± 0.84^{d}	58.55 ± 0.98^{d}
Test diet-II	1	57.34 ± 0.93^{c}	60.53 ± 0.84^{c}	64.17 ± 0.98^{b}
Test diet-III	2	70.81 ± 0.93^{a}	72.42 ± 0.90^{a}	70.44 ± 0.97^{a}
Test diet-IV	3	65.48 ± 0.61^{b}	64.02 ± 0.93^{b}	61.73 ± 0.88^{c}
Test diet-V	4	56.68 ± 0.68^{c}	59.44 ± 0.92^{c}	58.53 ± 0.58^{d}
Test diet-VI	5	46.48 ± 0.87^{e}	$48.84\pm0.89_{e}$	50.60 ± 0.87^{e}

Means within rows having different superscripts are significantly different ($p<0.05$) Data are means of three replicates

1984) and deficiency of Se was found to result in retarded growth. Mortality was observed in salmon fry fed a diet which is deficient in Se and mortality was prevented by the administration of a diet containing 0.1 mg Se kg^{-1} and 500 IU vitamin E kg^{-1} (Poston *et al.,* 1976). Growth performance of *C. auratus gibelio* was found to increase when fed on Se treated diet (Wang *et al*., 2007). Diet supplemented with nano Se showed improvements in the final weight and relative weight gain in *C. auratus gibelio* (Zhou *et al*., 2009). Se was found to be efficiently absorbed by juvenile rainbow trout from their diet, when fed on diet supplemented at different levels of selenium (0.2, 1.3 and 3.7 pg g^{-1}) for 24 weeks (Hilton and Atkinson, 1982).

The present study provided sufficient evidences that 2 mg kg^{-1} Se supplementation had a significant effect on the growth performance and nutrient digestibility of *C. carpio* fingerlings fed on plant meal based diets. Nano Se supplementation in SFM based diets may decrease the need for supplementing nutrients, which will reduce the cost of fish feed and nutrient's discharge through faeces into the aquatic ecosystem leading to environment friendly aquaculture.

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