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Dietetic impact of selenium nanoparticles (Se-Nps) on physiological factors of grass carp (*Ctenopharyngodon idella*) fishlings

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The experiment was conducted for 3 months duration from 10th September 2018 to 26th November 2018. The current study was carried out to investigate the dietary impacts of selenium nanoparticles and *Prosopis cineraria* on physiological parameters of *Ctenopharyngodon idella* fishlings. Sustaining a protein level, different trial diets complemented with Se-NPs and *Prosopis cineraria* were fed to groups of fish. The experiment was conducted in a semi-static condition consisting of 4 tanks. The test diet was fed at the rate of 3% of wet body weight (g) of fingerlings. On a fortnightly basis, weight (g) and total lengths (cm) were measured. At the end of the experiment, blood samples from fish in each group were collected and subjected to different hematological parameters. Physicochemical parameters of water analysis were calculated on a fortnightly basis by water analysis test throughout the experimental period. The G4 (Se-Nps and pods of *Prosopis cineraria*) showed significantly ($p \leq 0.05$) high mean values of weight (12.84 ± 0.23 g), Standard Length (10.03 ± 0.15 cm), high RGR (79.62 ± 6.41), high RBC values (2.8 ± 0.04), high WBC value (168.73 ± 6.03) and high value of PCV (45.81 ± 4.16). No mortality was observed during the trial. Therefore dietary Se-NP complementation up to the pace of 0.70 mg kg^{-1} diet has incredibly positive effects on the physio biochemical health phases of juveniles.

Keywords: dietary effects; selenium nanoparticles; hematology; grass carp; growth

INTRODUCTION

Fishes will eat nourishment containing NMs and nanotechnology could be utilized to enhance the conveyance of micronutrients or flimsy fixings in water bolsters. Fish eat feed containing nonmaterials; in this manner, nanotechnology can be utilized for enhancing the physiological and biochemical status of fish (Handy, 2012).

Selenium is associated with growth hormone emission, as selenium is a critical constituent of the deiodinase chemical, which is important for the correct working of the thyroid hormones (Kohrle and Gartner, 2009). So, selenium is

associated with growth hormone emission. Selenium additionally assumes a profitable job in the physiology of fish by enhancing the physiological status of the creature (El-Hammady *et al.*, 2007).

P. cineraria develop in dry and parched locales of Arabia and in areas of Pakistan mainly Punjab, deserts of this territory. (Malik and Kalidhar, 2007). Leaf concentrates of *P. cineraria* have been accounted for to indicate antibacterial, antihyperglycaemic, antihyperlipidemic, and antioxidative exercises (Velmurugan *et al.* 2011).

The grass carp (*Ctenopharyngodon idella*) is the type of fish with the biggest detailed creation in aquaculture internationally, more than five million tons for each year. The main point of this investigation is to enhance the physio biochemical wellbeing status of *Ctenopharyngodon idella* and to build its generation rate at the hatchery level as it is critical fish from Pakistani waters and to shield it from distressing conditions.

MATERIALS AND METHODS

The research was performed in the Laboratory of the Zoology department of Islamia University of Bahawalpur Bagdad ul jaded campus. Materials used for synthesis were Selenium dioxide (SeO₂) as a precursor and Hydrazine hydrate (NH₂NH₂) as a reducing agent (reducing selenium dioxide into elemental selenium particles). The precursor arrangement of SeO₂ (45-50 mM) was set up by dissolving 5.50-5.55 g of SeO₂ in 1000-1100 mL of refined water. Arrangement of hydrazine hydrate was then arranged by dissolving 6.20-6.30 mL of hydrazine hydrate in 500-550 mL of refined H₂O. At that point, under consistent mixing, hydrazine hydrate arrangement was added drop by drop to the precursor arrangement anticipating precipitates shaped. The reaction blend was then kept at room temperature for 2-3 days or until every one of the precipitates (nanoparticles) was settled; the blend was stressed through the filtration procedure, and particles were washed twice with refined water so all deposits were evacuated. The particles were kept in a heater medium-term and were dried at 100-110 °C. The dried particles were ground and sifted until the point that a fine powder was made. The fine powder was

portrayed by the X-beam diffraction (XRD) strategy; the XRD example of Se-NPS appears in Figure 1.

The feeding trial comprised of 75 days and was directed in 4 tanks in semi-static conditions getting oxygenated freshwater. Uniform-sized fish paying little heed to sex, thirty fish for every tank were supplied. One gathering of fish was sustained the basal protein diet (Table 1), while the other gathering of fish was bolstered an eating regimen enhanced with Se-NPs at various rates, the third tank encouraged with p. cineraria and the fourth tank was encouraged with both p. cineraria in addition to Se-NPs. At first, the fish have sustained their separate eating regimens at a rate of 3% of wet body weight, two times every day, and after that, the nourishing rate was balanced fortnightly. Day by day feed admission was recorded by evacuating undigested feed and defecation through siphoning. Tanks were cleaned every 15 days so as to counteract parasitic and algal growth in the tanks. Amid the bolstering preliminary, water quality parameters were estimated routinely, for example, water temperature and pH, which ran from 20 to 25 °C and from 7 to 8, individually. At the end of the experiment, blood samples from fish in each group were collected by cardiac puncture and were immediately transferred to EDTA coated tube. The samples were subjected to different hematological parameters. RBC, WBCs, and platelets count, with hemoglobin and packed cell volume (PCV). Weight (g) was measured in (g) by Electronic compact scale (SF-400A) individually Total length (cm) was calculated by scale (in cm) which increases on a fortnightly basis.

Table 1: Formulation of protein basal diet

Ingredients						
White fish food,	Soybean food	Sunflower food	Wheat fiber	Wheat flour	Canola oil	Vitamin C

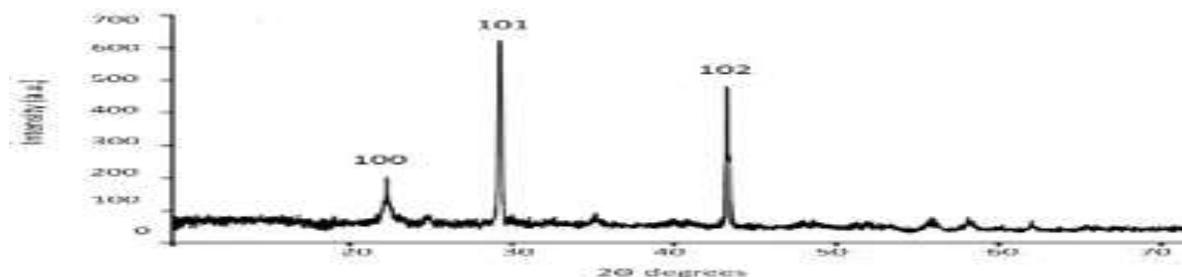


Figure 1: XRD model of Se-NPs

Total length (T.L.) was measured (in cm) by scale. FCR (feed conversion ratio), SGR (Specific growth rate), and S.R. (Survival rate) were also measured. All data were analyzed using STATISTICA 8.1 analytical software.

RESULTS

In table 2, the mean weights of *Ctenopharyngodon idella* fingerlings in (G1-G4) with different concentrations of Se-NPs and *p. cineraria* on fortnightly basis were given. The highest weight gain had shown in the G4 group in which fish had given selenium nanoparticles and *p. cineraria* both diets. Weight gain was also high in the G2 group in which diet was selenium nanoparticles. It can be observed that selenium nanoparticles had significant effects on the weight of fishes. *Prosopis cineraria* also had significant effects on the weight of fishes. In figure 2, the mean weights of *Ctenopharyngodon idella* fingerlings in (G1-G4) could be seen it could be observed that the highest weight gain was in G4 and lowest in G1. It can be said that diet containing SE-NPs and *p. cineraria* cause significant effects on fish physiology.

In table 3, ANOVA of mean weights of *Ctenopharyngodon idella* fingerlings can be seen. Values were significant at p-value which is 0.4. ANOVA between groups and within groups could be observed. In table 4, it could be seen that the survival rate is 89% in the control group. The survival rate in G2, G3, and G4 groups is

100%. RGR i.e. relative growth rate is 52.75, 78.58, 77.52, and 79.62 for G1, G2, G3, and G4 groups respectively. FCR i.e. feed conversion ratio is 1.73, 1.63, 1.65, and 1.64 for G1, G2, G3, and G4 groups respectively. In table 4 Mean lengths of *Ctenopharyngodon idella* fingerlings in (G1-G4) with different concentrations of Se-NPs and *p. cineraria* on fortnightly basis could be observed.

In figure 3, the mean lengths of *Ctenopharyngodon idella* fingerlings in (G1-G4) could be seen it could be observed that the highest length was in G4 and the lowest in G1. It can be said that diet containing SE-NPs and *p. cineraria* cause significant effects on fish physiology. In table 6, ANOVA of mean lengths of *Ctenopharyngodon idella* fingerlings can be seen. Values were significant at p-value which is 0.4. ANOVA between groups and within groups could be observed. In table 5 Hematological profiles (mean± SE) of fish exposed to different concentrations of Se-NPs and *p. cineraria* are given. In figure 4, RBCs of *Ctenopharyngodon idella* fingerlings in (G1-G4) could be seen it could be observed that the highest value was in G4 and the lowest in G1. It can be said that diet containing SE-NPs and *p. cineraria* cause significant effects on fish hematological profiles. It can be said that diet containing SE-NPs and *p. cineraria* cause significant effects on fish hematological profile.

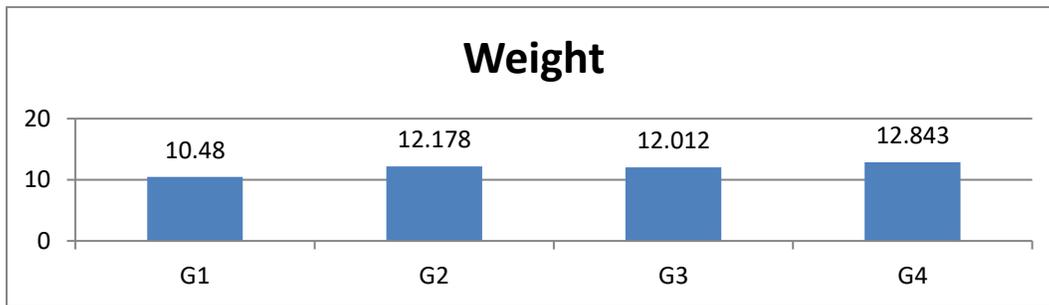


Figure 2: Mean weights of *Ctenopharyngodon idella* fingerlings in (G1-G4)

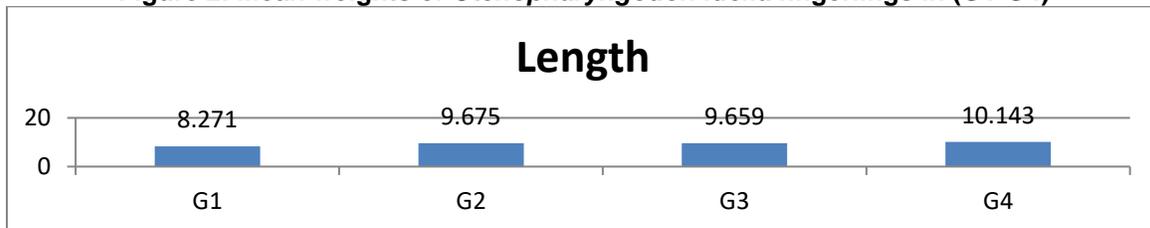


Figure 3: Mean lengths of *Ctenopharyngodon idella* fingerlings in (G1-G4)

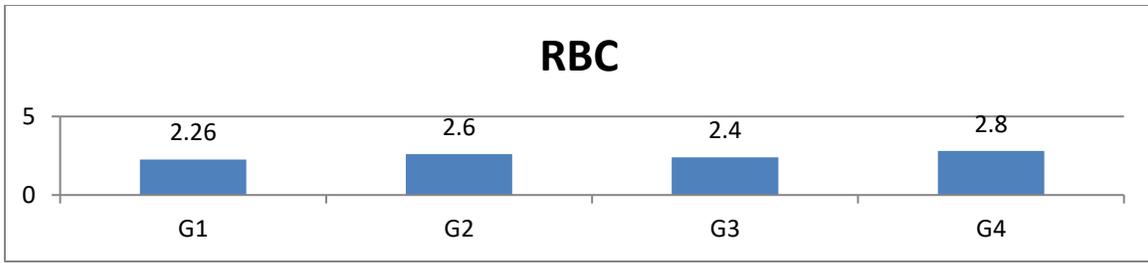


Figure 4 RBCs level of *Ctenopharyngodon idella* fingerlings in (G1-G4)

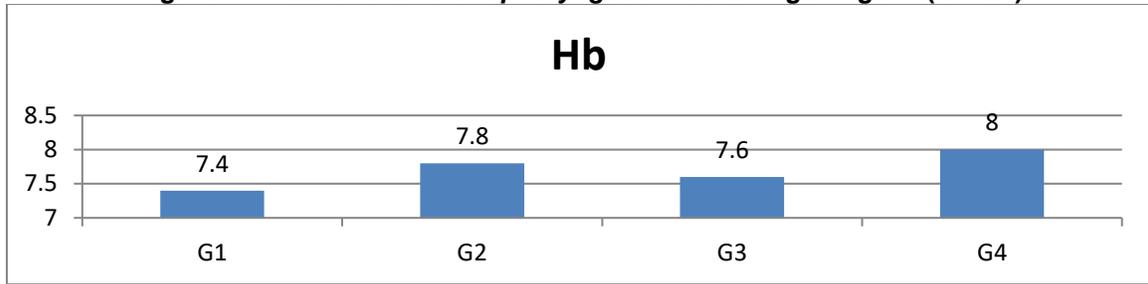


Figure 5 Hb levels of *Ctenopharyngodon idella* fingerlings in (G1-G4)

Table 2: Mean weights of *Ctenopharyngodon idella* fingerlings in (G1-G4) with different concentrations of Se-NPs and *p. cineraria* on fortnightly basis

Group No:	Initial weigh	1st fort night	2nd fort night	3rd fort night	4th fort night	5th fort night
G1(Control)	4.823	5.312	6.519	7.934	8.063	10.480
G2(Se-NPs)	4.876	6.787	8.246	9.385	10.476	12.178
G3(<i>p. cineraria</i>)	5.011	6.767	8.177	9.213	10.307	12.012
G4(Se-NPs + <i>p. cineraria</i>)	5.003	6.995	8.716	9.614	10.854	12.843

Table 3: ANOVA of mean weights of *Ctenopharyngodon idella* fingerlings

ANOVA Summary					
Source	Degrees of Freedom DF	Sum of Squares SS	Mean Square MS	F-Stat	P-Value
Between Groups	3	10.5507	3.8502	0.6115	0.4154
Within Groups	20	115.9272	6.2964		
Total:	23	125.4779			

Table 4: Parameters of *Ctenopharyngodon idella* fingerlings in (G1-G4)

Groups	Initial weight	Final weight	Survival rate	RGR%	FCR
G1(Control)	4.823	10.480	89%	52.75±5.66	1.73±0.10
G2(Se-NPs)	4.876	12.178	100%	78.58±7.4	1.63±0.10
G3(<i>p. cineraria</i>)	5.011	12.012	100%	77.52±13.41	1.65±0.11
G4(Se-NPs + <i>p. cineraria</i>)	5.003	12.843	100%	79.62±6.41	1.64±0.13

Table 5: Mean lengths of *Ctenopharyngodon idella* fingerlings in (G1-G4) with different concentrations of Se-NPs and *p. cineraria* on fortnightly basis

Group No:	Initial length	1 st fort night	2 nd fort night	3 rd fort night	4 th fort night	5 th fort night
G1(Control)	5.724	6.159	6.859	7.563	7.887	8.271
G2(Se-NPs)	5.730	6.168	7.096	8.231	8.948	9.675
G3(<i>p. cineraria</i>)	5.741	6.157	7.001	8.213	8.785	9.659
G4(Se-NPs + <i>p. cineraria</i>)	5.738	6.417	7.734	8.896	9.387	10.143

Table 6: ANOVA of mean lengths of *Ctenopharyngodon idella* fingerlings

ANOVA Summary					
Source	Degrees of Freedom DF	Sum of Squares SS	Mean Square MS	F-Stat	P-Value
Between Groups	3	2.277	0.959	0.4333	0.4315
Within Groups	20	34.2633	2.2132		
Total:	23	36.1403			

Table 7: Hematological profile (mean± SE) of fish exposed to different concentrations of Se-NPs and *p. cineraria*

Groups	RBC(10 ⁹ /L)	WBC(10 ⁹ /L)	Platelets(10 ⁹ /L)	Hb(g/dL)	PCV%
G1(Control)	2.26±0.08	130.57±6.22	148.65±8.11	7.4±0.20	32.68±2.75
G2(Se-NPs)	2.6±0.09	152.57±4.63	140.39±31.76	7.8±0.10	40.90±4.61
G3(<i>p. cineraria</i>)	2.4±0.06	158.51±2.09	131.27±10.52	7.6±0.33	42.87±3.09
G4(Se-NPs + <i>p. cineraria</i>)	2.8±0.04	168.73±6.03	124.6±9.64	8.0±0.13	45.81±4.16

DISCUSSION

Dietary effects of selenium nanoparticles and jhand plant (*Prosopis cineraria*) on physiological parameters of grass carp *Ctenopharyngodon idella* fish lings were determined in 75 days trial. The research was performed in the Laboratory of Zoology department of Islamia University of Bahawalpur Bagdad ul jaded campus. Se-NPs were synthesized via the precipitation method. The trial was performed in a semi-static condition consisting of 4 tanks. At the end of the experiment, blood samples from fish in each group were collected to determine hematological parameters (RBC, WBCs, and platelets count,

with hemoglobin and packed cells volume PCV). Weight (g) and Total length (cm) was also calculated. FCR (feed conversion ratio), SGR (Specific growth rate), and S.R. (Survival rate) were also measured. After applying the water test different parameters were calculated e.g. Temperature, pH, dissolved oxygen, alkalinity, hardness, and total dissolved solids.

During the study, no mortality was observed during the trial. Mean standard body weight was 4.823 to 10.480, 4.876 to 12.178, 5.011 to 12.012, and 5.003 to 12.843 in G1, G2.G3, and G4 groups respectively. Bodyweight was 5.724 to 8.271g, 5.730 to 9.675, 5.741 to 9.659, and 5.738 to 10.143 in G1, G2.G3, and G4 groups respectively

at the end of the experiment (Table 2 and Table 5). At the point when provided in feed at an ideal dimension, selenium has numerous advantageous consequences for the physiological and biochemical wellbeing of creatures, including fish (Jamil, 2013). Selenium has various organic capacities in creatures including fish (Kohrle *et al.*, 2000). It can be observed that the highest weight gain had shown in the G4 group in which fish had given selenium nanoparticles and *p. cineraria* both diets. Weight gain was also high in the G2 group in which diet was selenium nanoparticles. It can be said that selenium nanoparticles had significant effects on the weight of fishes. These results match with the work of Xuxia Zhou *et al.*, 2009 that clearly indicated that the Se supplemented diet could improve the final weight and relative gain rate of crucian carp, *Carassius auratus gibelio*. Selenium is also an essential trace element for the normal physiological function of growing animals (Brown *et al.*, 2001).

The mean lengths of *Ctenopharyngodon idella* fingerlings in (G1-G4) could be seen, it could be observed that the highest length was in G4 and lowest in G1. It can be said that diet containing SE-NPs and *p. cineraria* cause significant effects on fish growth. Gatlin & Wilson (1984) also demonstrated that the growth of channel catfish (*Zctalurus punctatus*) was affected by dietary Se level. Nano selenium causes good effects on the weight and growth of fish. Fish group that fed with special diets and that fed with only protein diets had clear differences in weight and length of fishes. Nano-selenium and selenomethionine supplemented with basal diet to crucian carp (*Carassius auratus gibelio*) improved the relative gain rate, the final weight of the fish (Zhou *et al.* 2009). The survival rate is 100% all over the trial while 89% in the control group. The relative growth rate is highest in the G4 group (Table 6).

Selenium is a solid antioxidant micronutrient; this solid antioxidant property of Se may give steadiness and uprightness of cells inside the creature's body and shield them from hemolysis. It was seen that RBC include was higher the blood of Nile tilapia nourished reviewed dimensions of a Se-enhanced eating regimen (0.5 to 4 mg kg⁻¹ eats fewer carbs) when contrasted with those raised on a without se diet. In this way, it has been demonstrated that selenium supplementation in fish feed builds the solidness of hematological parameters. (Molnár *et al.*, 2011). Hematocrit esteem was altogether

diminished when cross breed tilapia was raised on an eating routine not enhanced with Se or with a diminished dimension of Se (El-Hammady *et al.* 2007). As per these outcomes, the present research thinks about has additionally uncovered that an eating routine enhanced with Se-NPs fundamentally expanded RBC tally, WBC check, and Hb, estimations of *Ctenopharyngodon idella* when contrasted with the basal eating regimen.

RBC includes was most reduced control gathering and most astounding in G2 and G4. Hemoglobin level was likewise expanded in gatherings that bolstered with selenium nanoparticles (Table 7). WBC count indicates the immunity level. It could be observed that there were significant differences in WBC count in the control group vs. other groups. THE highest WBC count was in G4, so it can be said that selenium and *p. cineraria* cause significant effects on immunity and resistance against harmful substances in fish. Research studies have proved the role of selenium in improving the hematological indices of fish (Molnár *et al.*, 2011)

CONCLUSION

Taking everything into account, this research had exhibited that distinctive Se-NPs enhanced in basal diet could enhance the length, weight, relative gain rate, and hematological profile of *Ctenopharyngodon idella*. Every one of these parameters indicated a close relationship with dietary Se-NP supplementation. Moreover; nano-Se had all the characteristics of being increasingly compelling when joined with a plant diet of *p. cineraria*. It essentially builds ($P < 0.05$) the physiological parameters of the fish. The outcomes obviously demonstrated the constructive outcomes of dietary Se-NPs, in this manner proposing that Se-NPS up to the rate of 0.70 mg kg⁻¹ in the diet are satisfactory for the enhancement of the physiochemical wellbeing of juveniles *Ctenopharyngodon idella*.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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