Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Effects of Se nanoparticles supplementation on growth performance, hematological parameters and nutrient digestibility of *Labeo rohita* fingerling fed sunflower meal based diet

Efeitos da suplementação de nanopartículas de Se no desempenho de crescimento, parâmetros hematológicos e digestibilidade de nutrientes da dieta baseada em farinha de girassol alimentada com alevinos de *Labeo rohita*

N. Ahmad[a]*, S. M. Hussain[b], S. M. Azam[c], M. M. Shahzad[c], A. Noureen[d], R. Yaqoob[c], M. Lateef[c], A. Yawer[e], D. Riaz[c], A. Usman[f], M. Faizan[g], S. Hassan[h], A. Ishtiaq[h], P. Riaz[h], A. Ali[h], F. Amin[i], M. Imran[j], R. Kausar[k], M. Ahmed[l], W. Bashir[m], M. Adnan[n], A. Siddique[o], M. Farooq[p] and S. Ahmad[h]

[a]University of Jhang, Department of Zoology, Punjab, Pakistan
[b]Government College University, Department of Zoology, Fish Nutrition Lab, Faisalabad, Pakistan
[c]University of Education Lahore, Division of Science and Technology, Department of Zoology, Punjab, Pakistan
[d]The University of Lahore, Institute of Molecular Biology & Biotechnology – IMBB, Department of Zoology, Lahore,Pakistan
[e]Racetox, Masaryk University, Faculty of Science, Kamenice, Brno Czech Republic
[f]Government College University, Department of Chemistry, Faisalabad, Pakistan Lahore, Punjab, Pakistan
[g]University of Agriculture, Department of Zoology, Faisalabad, Punjab, Pakistan
[h]Institute of Pure & Applied Biology Bahauddin Zakariya University, Multan, Pakistan
[i]University of Veterinary & Animal Sciences Punjab, Department of Zoology, Lahore,Pakistan
[j]BahuddinZakariya University Multan, Department of Statistics, Punjab, Pakistan
[k]University of Baluchistan, Department of Zoology, Quetta, Pakistan
[l]COMSATS University Islamabad, Department of Management Sciences, Vehari Campus, Vehari, Pakistan
[m]Department of Zoology, Government College University, Faisalabad, Pakistan
[n]Department of Zoology, Government Graduate Taleem-ul-Islam College Chenab Nagar, Chiniot, Pakistan
[o]Department of Chemistry, Lahore College for Women University Lahore, Pakistan
[p]Department of Zoology, Ghazi University Dera Ghazi Khan, Pakistan

Abstract

The aim of the present study is to assess the effects of selenium nanoparticles on the growth, hematology and nutrients digestibility of *Labeorohita* fingerlings. Fingerlings were fed with seven isocaloric sunflower meal-based diet supplemented with different concentrations of nanoparticles naming T_1 to T_7 (0, 0.5, 1, 1.5, 2, 2.5, and 3 mg/kg), with 5% wet body weight while chromic oxide was used as an indigestible marker. After experimentation for 90 days T_3 treated group (1mg/kg $^{-1}$Se-nano level) showed the best result in hematological parameters (WBC's $7.97 \times 10^3 mm^{-3}$, RBC's 2.98×10^6 mm^{-3} and Platelet count 67), nutrient digestibility (crude protein: 74%, ether extract: 76%, gross energy: 70%) and growth performance (weight gain 13.24 g, weight gain% 198, feed conversion ratio 1.5, survival rate 100%) as compared to the other treatment groups. Specific growth rates were found significantly higher in T_5 than in other groups. The present study indicated positive effect of 1 mg/kg Se-nanoparticles on growth advancement, hematological parameters, and nutrients digestibility of *L. rohita* fingerlings.

Keyword: selenium nanoparticle, hematology, nutrients digestibility, *L. rohita*, growth.

Resumo

O objetivo do presente estudo é avaliar os efeitos das nanopartículas de selênio no crescimento, hematologia e digestibilidade dos nutrientes de alevinos de *Labeo rohita*. Os alevinos foram alimentados com sete dietas isocalóricas à base de farinha de girassol suplementada com diferentes concentrações de nanopartículas, nomeando T1 a T7 (0, 0,5, 1, 1,5, 2, 2,5 e 3 mg / kg), com 5% do peso corporal úmido enquanto o óxido crômico foi usado como

*e-mail: drnisarahmad@uoj.edu.pk
Received: June 23, 2021 – Accepted: August 30, 2021

um marcador indigesto. Após a experimentação por 90 dias, o grupo tratado com T3 (nível 1mg / kg -1Se-nano) mostrou o melhor resultado em parâmetros hematológicos (WBC's 7,97 × 103mm-3, RBC's 2,98 × 106mm-3 e contagem de plaquetas 67), digestibilidade dos nutrientes (proteína bruta: 74%, extrato de éter: 76%, energia bruta: 70%) e desempenho de crescimento (ganho de peso 13,24 g, ganho de peso % 198, taxa de conversão alimentar 1,5, taxa de sobrevivência 100%) em comparação com os outros grupos de tratamento. As taxas de crescimento específicas foram encontradas significativamente mais altas em T5 do que em outros grupos. O presente estudo indicou efeito positivo de 1 mg / kg de nanopartículas de Se no avanço do crescimento, parâmetros hematológicos e digestibilidade de nutrientes de alevinos de *L. rohita*.

Palavras-chave: nanopartícula de selênio, hematologia, digestibilidade de nutrientes, *L. rohita*, crescimento.

1. Introduction

Fish flesh contains the highest quality of protein, nutrients, and vitamins that our body needs, and worldwide animal protein consumption is 17% (Kwasek et al., 2020; Shah and Mraz, 2020). In south India, *L. rohita* is the most demanding fish and with *Catla catla* and *Cirhinus mrigala* it contributes 67% of fresh water production (Krishnaveni et al., 2013).

Selenium is considered to be avital micronutrient for the regular performance of the body and its supplemented diet could play an important role to enhance the quality parameters like growth, hematology and nutrient digestibility (Handy et al., 2012). It also boosts immune system activities. Various studies recommend the use of selenium nanoparticles as a dietary source. Selenium deficient diet in organisms can cause problems like pneumonia, infertility, and oxidative stress (Pelyhe and Miklos, 2013). Nanoparticles have a wide range of applications that help in improving feed quality, absorption of nutrients, and utilization of minerals (Vijayakumar and Balakrishnan, 2014).

The demand for food especially proteins is increasing day by day as the human population has been increased. To overcome this problem aquaculture is the best option to achieve the goal of fulfillment of this demand of proteins. New technologies in aquaculture have made it cost-effective and helping to create a friendly and healthy environment for the aquatic organism (Hussain et al., 2015a). Fish meal is an expensive source of protein and nutrients in feed. About 60% of the total cost of the fish culture is paid in feed formulation (Essa et al., 2004).

Scientists used plant byproducts as the source of protein and nutrients in fish feed over the last few decades (Hussain et al., 2015a). In the current study, we selected sunflower meals as an alternative source of protein and nutrients. In Pakistan, sunflower meal is preferred as a diet for aquaculture as it contains proteolytic enzymes and has the lowest cost (Khan et al., 2006). It has 45-48% crude protein. It is a palatable and nutritionally balanced diet to achieve maximum fish growth results (Tahir et al., 2008). Sunflower is also the best, cheap, and easily available source of proteins (Mushtaq et al., 2006) as compared to fish meal.

The main objective of this study was to calculate the effect of Se nano-particles supplemented diet on growth, nutrients digestibility and hematology of *L. rohita* fingerlings.

2. Materials and Methods

Healthy fingerlings of *L. rohita* were brought and acclimatized in Fish Nutrition Lab, Government Collage University Faisalabad from Govt. Fish Seed Hatchery, Faisalabad for 15 days and fed with basal diets (Allan and Rowland, 1992).

2.1. Feed ingredient analysis and selenium nanoparticle

Analysis of feed ingredients was done by using the standard methods (AOAC, 1995). Nanoparticles of Se were analyzed with TEM and XRD (TEM.JEOL2100.20171206) (Iqbal et al., 2014) for confirmation of size and structure as pure crystalline because they were purchased from the market (Sigma Aldrich).

2.2. Pellets formation

Feed pellets were formulated by following Lovell, 1989. Feed ingredients were grounded to a size of 0.5mm and pass through a sieve of 0.5mm to confirm the grain sizes. All ingredients were then mixed for 5 min with the gradual addition of fish oil. Water was added to make suitable dough after mixing of ingredients and pellets of the desired size were formulated thereafter.

2.3. Preparation of stock solutions of NP's

Protocols given by Federici et al. (2007) were followed properly for preparing and conformation of stock solutions of nanoparticles. Sonication method (for 6-8Hr) was used to prepare the stock solution of dry powder of pure NPs and further dilutions were prepared from these stock solutions to ensure the required level (0, 0.5, 1, 1.5, 2, 2.5 and 3mg/Kg) of Se NPs.

2.4. Adding nanoparticles to basal diet

Just before spraying the diluted Se solutions on the basal diet, they were sonicated for extra 15 minutes as recommended by Ramsden et al.(2009). Required concentration was sprayed gradually after pouring one Kg feed into food mixer. The seven test isocaloric and isoenergetic diets were prepared by spraying classified levels (0, 0.5, 1, 1.5, 2, 2.5 and 3 mg/Kg) of nanoparticles of Se which coated with feed pellet immediately. Feed pellet was dried and then eventually stored in an airtight container for future use (Table 1 and 2) .

2.5. Sample collection and feeding protocol

Each control as well as an experimental group comprised of triplicates with 15 fingerlings which were fed for 90 days

Table 1. Feed ingredients with percent chemical composition.

Ingredients	Fish meal	Rice polish	Wheat flour	Sunflower
%Dry matters	91.67	94.06	92.4	93.80
%Crude Protein	49.03	11.87	09.73	40.81
%Crude Fat	6.93	12.69	2.24	3.69
%Crude Fiber	1.23	11.91	2.73	1.94
%Ash	23.15	11.32	1.99	09.96
Gross Energy(kcal/g)	2.49	3.41	3.06	3.64
Carbohydrate	19.66	52.21	82.21	43.6

Table 2. Percent composition of ingredients oil seed meal based test diets.

Ingredients	Test Diet						
	I	II	III	IV	V	VI	VII
Nanoparticles (mg/kg)	0	0.5	1	1.5	2	2.5	3.0
Sunflower meal	50 for all test diets						
Fish meal	14.5for all test diets						
Wheat flour	13 for all test diets						
Rice polish	11 for all test diets						
Fish oil	7.5 for all test diets						
Vitamin premix	1 for all test diets						
Minerals premix	1 for all test diets						
Ascorbic acid	1 for all test diets						
Chromic oxide	1 for for all test diets						

with different above-said concentrations. Feces were collected from each tank by opening and closing of valves following Hussain et al. (2018).

2.6. Feces and feeds analysis

Standardized methods by AOAC (1995) were used to analyze feed ingredients, feces, and test diets. Crude fat and crude fiber were determined from micro-Kjeldahl apparatus and oxygen bomb calorimeter in test diets and feces. Chromic oxide estimation in feces and diets was donewith a spectrophotometer (Model: UV.VIS 2001) at an absorbance of 370nm (Divakaran et al., 2002).

2.7. Study of growth

Fingerling growth performance was estimated by using the standard method as represented by Hussain et al. (2015b). As Percentage weight gain is determined by using Equation 1; FCR by Equation 2 and % SGR by Equation 3.

$$\%\ Weight\ gain = \frac{Final - Initial\ weight}{Initial\ weight} \times 100 \quad (1)$$

$$Feed\ conversion\ ratio = \frac{Total\ intake\ of\ dry\ feed}{Wet\ body\ weight\ gain} \quad (2)$$

$$\%Survival\ growth\ rate = \frac{Final - Initial\ fish\ weight}{Trial\ day} \times 100 \quad (3)$$

2.8. Nutrient digestibility

Apparent coefficient for nutrient digestibility (ACD) in experimentation diet was determined by using formula represented by Hussain et al. (2015a). Percentage ADC determined by using Equation 4.

$$\%ADC = 100 - 100 \times \frac{\%\ nutrient\ in\ feces \times \%\ diet\ marker}{\%\ nutrient\ in\ diet \times \%\ feces\ marker} \quad (4)$$

2.9. Hematological parameter

Hematological parameters were calculated by protocols of Peake (1998) and Coyle et al. (2004). The capillary tube method was used to determine the hematocrit (Brown, 1988). WBC's and RBC's were calculated with a haemocytometer. The concentration of Hemoglobin was evaluated as defined by Blaxhall and Daisley (1973). To calculate MCHC; MCH and MCV following formulae were used in Equation 5, 6 and 7:

$$Mean\ corpuscular\ hemoglobin\ concentration\ (MCHC) = Hb / PCV \times 100 \quad (5)$$

$$Mean\ corpuscular\ hemoglobin\ (MCV) = PCV / RBC \times 10 \quad (6)$$

$$Mean\ cell\ volume\ (MCH) = Hb / RBC \times 10 \quad (7)$$

2.10. Statistical analysis

All the experimental data were analyzed using ANOVA (Steel et al., 1996) followed by Tukey's Honesty method (Snedecor and Cochran, 1991). Statistical analyses were performed by using Co-Stat Package (Version 6.303USA).

3. Results

Transmission Electron Microscope and X-Ray Diffraction techniques were used for the confirmation of the size, shape, and phase composition of (Se NPs). TEM clarifies the round shaped particles of about 8-10nm which are homogenous in size. Figure 1 demonstrates the morphological analysis of Se-nanoparticles in magnification form as shown in Figure 1a and normal TEM in Figure 1b. In these figures, 10nm and 50nm scale bars were used for the magnified TEM and normal TEM images. This result shows that the size and nature of nanoparticles less than 100nm about 10nm and pure which are used in test diets. The X-ray Diffraction technique, confirms that the sample is in a nano-crystalline structure and the same as the standard selenium powder of these nanoparticles in Figure 2.

Hematological indices such as WBC's ($7.97\times10^{3}mm^{-3}$), RBC's ($2.98\times10^{6}mm^{-3}$), Platelet count (66.64), Packed cell volume (24.98%), hemoglobin concentration (8.72g/100ml) and Mean corpuscular volume (188.22fl) werefound at their best level in fish fed by the test diet supplemented with 1mg/kgSelenium nanoparticle diet (T_3) while, Mean corpuscular hemoglobin concentration (35.12pg) was observed at its best at 2mg/kg Se-nano level; (T_5) whereas mean corpuscular hemoglobin (58.22pg) was observed at its best level at 0.5mg/kg Se-nano level; (T_2). The data of hematological parameters aregiven in Table 3. The values of hematological parameters in the fish fed a control diet were as WBCs ($7.25\times10^{3}mm^{-3}$), RBCs $2.16\times10^{6}mm^{-3}$), PLT (58.39), PCV (23%), MCV (180.38fl), Hb (7.6g/100ml), MCH (52.51) and MCHC (31.94pg).

A significant difference($p<0.05$) of all the hematological parameters were observed between the fingerlings fed experimental diets and controlled one which indicates that Se-nano plays a crucial role in the growth and health of fish when supplemented in fish feed. The lowest values of WBCs ($6.75\times10^{3}mm^{-3}$), RBCs ($1.33\times10^{6}mm^{-3}$), PLT (55.29), and Hb (6.61g/100ml) were found at 2.5 Se-nano(mg/kg) level; (T_6) while PCV (21.62%), MCV (99.15fl), MCH (40.08) and MCHC (26.93pg) were detected in fed by fish to test diet (T_7) which was supplemented with 3 Se-nano(mg/kg).As can be seen from the above results, hematological parameters began to increase with an increase in nanoparticle concentration till 2mg/kg Selenium nanoparticle and then began to decrease with increase the supplementation of Se-nano.

The percentage of nutrients present in feces, feed, and digestibility are shown in Table 4, 5, and 6 respectively. In test diet (T_3) supplemented with 1 Se-nano(mg/kg), the coefficient of apparent digestibility (crude protein: 74%, ether extract: 76% and gross energy 70%) were also recorded at their highest level. While these values were observed to be significantly different ($p<0.05$) in the control group (crude protein: 53%, ether extract: 57%, and gross energy 53%). A highly significant difference (upto 21% crude protein, 19% ether extract and 17% gross energy) in nutrients digestibility of controlled and experimental levels can be observed. Lower value of digestibility of nutrient (crude protein: 51%, ether extract: 51% and gross energy

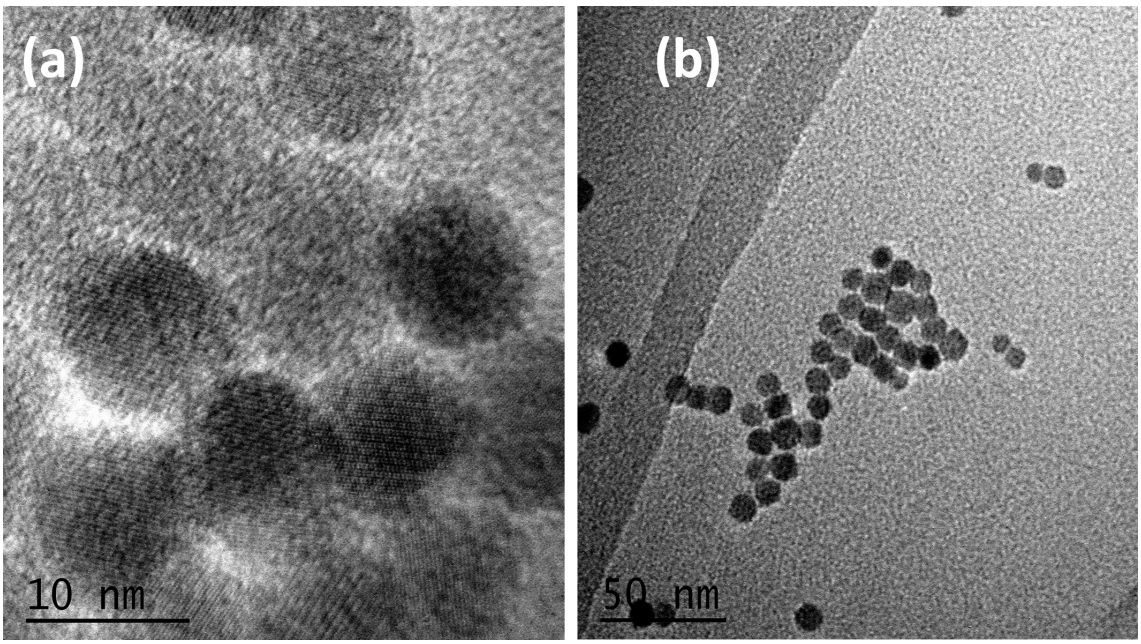

Figure 1. View of Selenium Nanospheres by Transmission Electron Microscopic (a) 10nm scale bar (b) 50nm scale bar with magnified form.

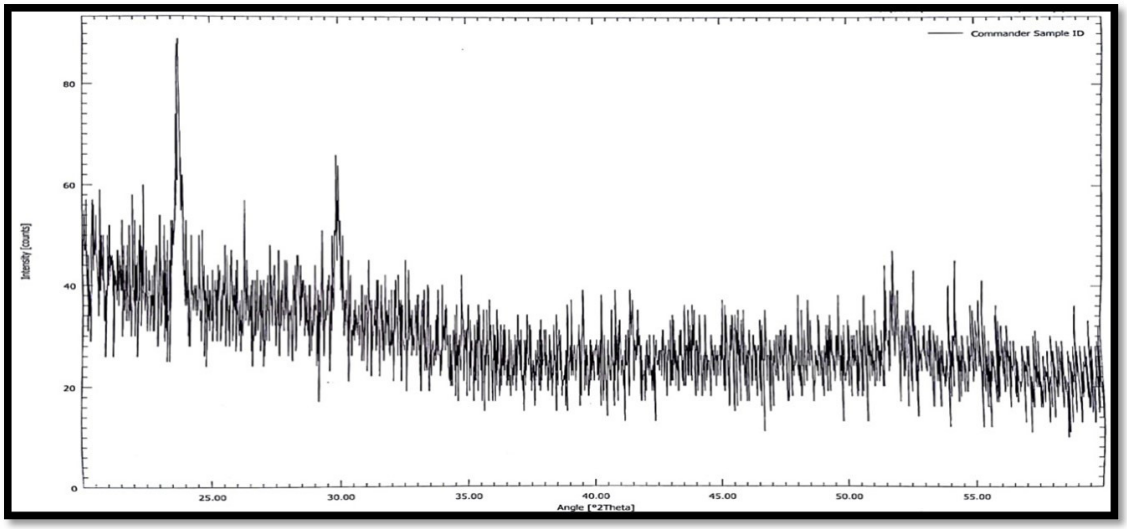

Figure 2. Selenium Nano-spheres view by X-Ray Diffraction.

Table 3. Hematological parameters of *L. rohita* fingerling graded level fed with Se-nano supplemented Sunflower meal based diets.

Diets	Se-nano (mg kg^{-1})	RBC (10^6mm^{-3})	WBC (10^3mm^{-3})	PLT	Hb (g/100ml)	PCV (%)	MCHC (%)	MCH (pg)	MCV (fl)
Test Diet –I (Control diet)	0	2.16^{c}	7.25^{b}	58.39^{c}	7.6^{bc}	22.99^{d}	31.94^{c}	52.51^{c}	180.38^{d}
Test Diet –II	0.5	2.65^{b}	7.71^{a}	63.30^{b}	8.08^{ab}	24.39^{abc}	35.25^{a}	58.22^{a}	181.86^{c}
Test Diet –III	1	2.98^{a}	7.97^{a}	66.64^{a}	8.72^{a}	24.98^{a}	33.96^{b}	52.41^{c}	188.22^{a}
Test Diet –IV	1.5	2.74^{ab}	7.83^{a}	64.01^{b}	7.8^{bc}	23.82^{bc}	32.51^{c}	44.41^{d}	186.19^{b}
Test Diet –V	2	2.66^{b}	7.72^{a}	63.82^{b}	8.14^{ab}	24.43^{ab}	35.12^{a}	57.05^{b}	181.79^{c}
Test Diet –VI	2.5	1.33^{e}	6.75^{c}	55.29^{e}	6.61^{d}	23.63^{cd}	29.52^{d}	52^{c}	178.94^{e}
Test Diet –VII	3	1.69^{d}	6.99^{bc}	57.25^{d}	7.25^{cd}	21.62^{e}	26.93^{e}	40.08^{e}	99.15^{f}
*PSE		0.06	0.07	0.18	0.14	0.16	0.22	0.21	0.23
P-Value		.0***	.0***	.0***	.0***	.0***	.0***	.0***	.0***

These are different abbriviation of Complete Blood Count in Table as: RBC (Red Blood Cell). WBC (White Blood Cell). PLT (Platelets). Hb (Hemoglobin). PCV (Packed Cell volume). MCHC (Mean Corpuscular Hemoglobin Concentration). MCH (Mean Corpuscular Hemoglobin). MCV (Mean Corpuscular Volume). a, b and c on values used to show the interreltion among these as Duncan test applied on it; while star pattern is used to notify the signification of parameters with diet as these notify highly significant correlation among these. *PSE = pooled SE = $\sqrt{MSE/n}$ (where MSE = mean-squared error). ***$p < 0.001$, P-value less than 0.0001 shows highly significant correlation.

49%) wererecorded at 2.5 Se-nano(mg/kg) level (T_6) and at 3 Se-nano(mg.kg) level (T_7) respectively. Parameters for nutrient digestibility began to increase with increasing the concentration of nanoparticle till 2mg/kgSe-nano but further increase above this mentioned level in Se-nano supplementation causes the decline in parameters values as observed from above-mentioned results.

The growth data is shown in Table 7 and the highest growth (13.24g), %weight gain (198), Feed conversion ratio (1.5), and with 100% survival were recorded in (T_3) at 1 Se-nano (mg/kg) diet, while best survival growth rate (1.55) in (T_5) at 2 Se-nano (mg/kg). The values of growth parameters in the control group were weight gain (10.95g), percentage weight gain (163), Feed conversion ratio (1.86), survival growth rate(1.08), and survival (98%). Highly significant differences (upto 2.29 of weight gain, 34% weight gain, 0.36 feed conversion ratio, 0.47 survival growth rate and 2% in survival were recorded.The lowest growth indices were observed as weight gain (10.08 g), percent weight gain (147), Feed conversion ratio (1.88), Survival growth rate(1.01), and survival (96%) in treatment group 7 (T_7) at 2.5 Se-nano (mg/kg) diet. This result shows that increment of Se-nano in the diet enhances the efficiency of growth parameters till 2 mg/kg, afterward decline with an additional increase of Se-nanoparticles in the diet.

4. Discussion

Blood is an indicator of the physiological condition health of the internal environment of any organism. Therefore, experiments were conducted to study the

Table 4. Percentage of nutrients in test diets of *L. rohita* fingerlings fed graded levels of Se-nano supplemented Sunflower meal based diets.

Diets	Levels	Se-nano(mg kg^{-1})	%CP in diet	%EE in diet	GE (kcal/g) in diet
Test Diet (Control diet)	I	0	31.40	6.94	3.87
Test Diets	II	0.5	31.49	6.94	3.87
	III	1	31.39	6.92	3.86
	IV	1.5	31.35	6.94	3.87
	V	2	31.43	6.92	3.88
	VI	2.5	31.36	6.92	3.86
	VII	3	31.40	6.91	3.86
	*PSE		0.19	0.029	0.035
	P Value		.998ns	.986ns	.999ns

*PSE = pooled SE = √MSE/n (where MSE = mean-squared error). CP (Crude protein). EE (Efficient energy). GE (Gross Energy). While 'ns' for non significant correlation as parameters with diet notify no effective correlation among these

Table 5. Percentage of nutrients in feces *of L. rohita* fingerlings fed graded levels of Se-nano supplemented Sunflower meal based diets.

Diets	Levels	Se-nano(mg kg^{-1})	% CP in feces	% EE in feces	GE (kcal/g) in feces
Test Diet (Control diet)	I	0	16^{a}	3.26^{b}	1.99^{b}
Test Diet	II	0.5	12.86^{b}	2.6^{c}	1.62^{cd}
	III	1	8.75^{c}	1.77^{e}	1.25^{e}
	IV	1.5	9.8^{c}	2.26^{d}	1.48^{d}
	V	2	12.69^{b}	2.69^{c}	1.71^{c}
	VI	2.5	16.73^{a}	3.27^{b}	2.10^{ab}
	VII	3	16.61^{a}	3.77^{a}	2.23^{a}
*PSE		0.255501	0.063032	0.045635	
P Value		.0***	.0***	.0***	

*PSE = pooled SE = √MSE/n (where MSE = mean-squared error). CP (Crude protein). EE (Efficient energy). GE (Gross Energy). a, b and c on values used to show the interreltion among these as Duncan test applied on it; while star pattern is used to notify the signification of parameters with diet as these notify highly significant correlation among these. ***p < 0.001, P-value less than 0.0001 shows highly significant correlation.

Table 6. Percentage of nutrients digestibility of *L. rohita* fingerlings fed graded levels of Se-nano supplemented Sunflower meal based diets.

Diets	Se-nano(mg/kg)	% CP digestibility	% EE digestibility	% GE digestibility
Test Diet –I (Control diet)	0	53.47^{e}	57.1^{d}	53.01^{e}
Test Diet –II	0.5	62.46^{d}	65.50^{c}	61.62^{c}
Test Diet –III	1	74.09^{a}	76.19^{a}	69.86^{a}
Test Diet –IV	1.5	71.80^{b}	70.65^{b}	65.51^{b}
Test Diet –V	2	63.79^{c}	65.13^{c}	60.45^{d}
Test Diet –VI	2.5	51.46^{f}	57.03^{d}	50.51^{f}
Test Diet –VII	3	52.83^{e}	51.39^{e}	48.63^{g}
*PSE		0.271132	0.168676	0.177
P Value		.0000***	.0000***	.0000 ***

*PSE = pooled SE = √MSE/n (where MSE = mean-squared error). CP (Crude protein). EE (Efficient energy). GE (Gross Energy). a, b and c on values used to show the interreltion among these as Duncan test applied on it; while star pattern is used to notify the signification of parameters with diet as these notify highly significant correlation among these. ***p < 0.001, P-value less than 0.0001 shows highly significant correlation.

Table 7. Growth performance of *L. rohita* fingerlings fed graded levels of Se-nano supplemented Sunflower meal based diets.

Diets	Levels	Se-nano(mg/kg)	Initial weight(g)	Final weight (g)	Weight gain(g)	Weight gain(%)	Feed Intake (g)	Weight gain (fish^{-1} day^{-1}) g	Feed Conversion Ratio	Specific Growth Rate	%Survival
Test Diet (Control diet)	I	0	6.7	17.64^{d}	10.95^{e}	163.46^{d}	0.23^{ab}	0.12^{e}	1.86^{a}	1.08^{e}	97.78^{a}
Test Diets	II	0.5	6.69	18.71^{c}	12.02^{d}	179.56^{c}	0.23^{a}	0.13^{d}	1.73^{bc}	1.14^{d}	97.78^{a}
	III	1	As	19.92^{a}	13.24^{a}	197.97^{a}	0.22^{ab}	0.15^{a}	1.50^{d}	1.19^{c}	100.00^{a}
	IV	1.5	As	20.01^{a}	12.78^{b}	191.19^{b}	0.23^{a}	0.14^{b}	1.61^{cd}	1.22^{b}	100.00^{a}
	V	2	As	19.20^{b}	12.51^{c}	187.04^{b}	0.22^{ab}	0.14^{c}	1.60^{d}	1.55^{a}	100.00^{a}
	VI	2.5	As	18.59^{c}	11.90^{d}	177.79^{c}	0.23^{a}	0.13^{d}	1.76^{ab}	1.14^{d}	97.78^{a}
	VII	3	6.85	16.93^{e}	10.08^{f}	147.12^{e}	0.21^{b}	0.11^{f}	1.88^{a}	1.01^{f}	95.56^{a}
	*PSE		0.042	0.093	0.055	1.076	0.0006	0.003	0.024	0.005	1.68

*PSE = pooled SE = √MSE/n (where MSE = mean-squared error). a, b and c on values used to show the interreltion among these as Duncan test applied on it; while star pattern is used to notify the signification of parameters with diet as these notify highly significant correlation among these.

hematological parameters of *L. rohita*. The following parameters were evaluated in blood: WBCs, RBCs, Platelets, Haemoglobin concentration, Packed cell volume and Mean corpuscular volume. Our findings show nanoparticles improve the hematological parameters, as previously proven by Behera et al. (2014) that iron nanoparticles can improve the hematological parameters of *L. rohita*. Hematological parameters for loach can be boosted up using nano-Se supplementation as reported by Hao et al. (2014). The results of El-Hammady et al. (2007) also supported our findings according to whom selenium supplementation improved the percentage of hematocrit and RBC count in hybrid tilapia. The results we obtained were quite similar to Khalafalla et al. (2011) and Le et al. (2013) as they previously described that Se can improve the immunity and RBCs count in the fish. Stability in hematological parameters may increase if the Selenium supplement is used in fish feed. The reasons behind the stability and integrity of cells in fish could be the strong antioxidant property of Se which also protects cells from hemolysis (Khan et al., 2016). The reproductive potency of lymphocytes is affected greatly if there is a deficiency of selenium which causes the altering in the transferring receptors. Selenium helps in the production and proliferation of antibodies and by activation of GSH-Px, it also protects the B lymphocytes. The activities of GSH-Px increased significantly ($p<0.05$) in plasma and tissue due to nanoparticles of selenium or selenomethionine which directly affect and improve the production of white blood cells in carps. Production and proliferation of B-lymphocytes improve the lysozymatic activities of fish. Activation of plasma lysozyme and expression of IgM is also stimulated by Se. The life span of WBCs and RBCs may increase in the response to antioxidant properties of nanoparticles (Alimohamady et al., 2013).

A difference of 34% in weight gain was observed in fingerlings who were fed on the control diet and experimental diet (T_3) 1mg/kgSe-nano. A continuous decline in growth parameters was observed when the higher levels of supplements were used; the reason might be the toxic effects of Se-nano. Other growth parameters like Survival growth rate, feed conversion ratio, and survival rate were also observed to be at their best at the experimental diet (T_3) with a significant difference ($p<0.05$) from the control diet. Our results are in general agreement with Ashouri et al. (2015), who found the positive effect of Se-nano supplementation in common carp. They used Se-nano levels of 0, 0.5, 1, and 2 mg/kgfor 2months and observed that percentage fish weight gain remained highest at 1 mg nano-Se/kg level yet all the levels of parameters were significantly better thancontrolledoneexcept survival parameter which was 100% in all four given treatments. Our findings are also similar to the results of Faiz et al. (2015), who found that there was a difference of 84% in weight gainof juveniles grass carp fed nano-ZnO than that of control one. The almost same trend was observed by Zhou et al. (2009) who reported that all growth parameters like weight gain, food conversion rate, and relative gain rate improved significantly in crucian carp fed nano-Se as compared to control one. However, the survival rate was the same in all treatments 100%. Selenim improves growth because it works as an active core of glutathione peroxidase. Deficiency of Selenium affects the activities of GSH-Px which ultimately leads to the reduction of lipidichydroperoxides as well as hydrogen peroxide at cellular levels (Zhou et al., 2009). The reason behind decreasing the carcass parameters, if supplied with higher levels of supplementation, is that when the concentration of nanoparticle that crosses above mentioned optimum level of feed start for palatability loss(Onuegbu et al., 2018).

The results given by Ramsden et al. (2009) were quite contradictory to our finding as they reported no significant effect on growth parameters of rainbow trout in case of exposing to TiO_2 nanoparticles. Another type of contradiction was observed by Lin et al. (2014) who find that weight gain decreased in broilers when nano-Cr was added in feed as compared to control one. Similarly, Wang et al. (2015) and Hassan et al. (2013), observed inhibition of growth in *Epinephelus coioides* and mold respectively when they were exposed to nano-particles of copper and zinc oxide.

The nano-Se are very important for the proper digestibility of nutrients in fingerlings of *L. rohita* as proven by our experiments. Our results are quite similar to Gonzales-Eguia et al. (2009) who also reported a positive increase in nutrients digestibility when nano-copper were fed to piglets as compared to control one. Similar results were also provided by Kumari et al. (2013) who reported that nutrients digestibility improves in *L. rohita* with the supplementation of encapsulated nano trypsin (0.01-0.02%). The reason for this advancement is the deposition mechanism and metabolism pathway of Se Nanoparticles in fish and because of this soluble proteins can interact with NPs to form a halo (cr). (Onuegbu et al., 2018).

In conclusion, we found that nano-Se supplementation, 1mg/kgSe-nano, in a sunflower meal-based diet stimulates growth performance, hematological indices, and digestibility of nutrients of *L. rohita* fingerlings. Its supplementation (at the above said best level) is very important for production of environment friendly fish feed.

Acknowledgements

This research work is done in Fish Nutrition lab, Department of Zoology, Government College University, Faisalabad. We are thankful to administration for their support in conduction of research work.

References

ALIMOHAMADY, R., ALIARABI, H., BAHARI, A. and DEZFOULIAN, A.H., 2013. Influence of different amounts and sources of selenium supplementation on performance, some blood parameters, and nutrient digestibility in lambs.*Biological Trace Element Research*, vol. 154, no. 1, pp. 45-54. http://dx.doi.org/10.1007/s12011-013-9698-4. PMid:23677850.

ALLAN, G.L. and ROWLAND, S.J., 1992. Development of an experimental diet for silver perch (Bidyanus bidyanus).*Austasia Aquaculture*, vol. 6, pp. 39-40.

ASHOURI, S., KEYVANSHOKOOH, S., SALATI, A.P., JOHARI, S.A. and PASHA-ZANOOSI, H., 2015. Effects of different levels of dietary selenium nanoparticles on growth performance, muscle

composition, blood biochemical profiles and antioxidant status of common carp (*Cyprinus carpio*).*Aquaculture*, vol. 446, pp. 25-29. http://dx.doi.org/10.1016/j.aquaculture.2015.04.021.

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS – AOAC, 1995. *Official methods of analysis*.15th ed. Washington, D. C.: AOAC.

BEHERA, T., SWAIN, P., RANGACHARULU, P.V. and SAMANTA, M., 2014. Nano-Fe as feed additive improves the hematological and immunological parameters of fish, *Labeo rohita H.Applied Nanoscience*, vol. 4, no. 6, pp. 687-694. http://dx.doi.org/10.1007/s13204-013-0251-8.

BLAXHALL, P.C. and DAISLEY, K.W., 1973. Routine haematological methods for use with fish blood.*Journal of Fish Biology*, vol. 5, no. 6, pp. 771-781. http://dx.doi.org/10.1111/j.1095-8649.1973.tb04510.x.

BROWN, B.A., 1988. *Hematology: principle and procedures*. Philadelphia: Lea &Febiger, Routine hematology procedures, pp. 7-122.

COYLE, S.D., DURBOROW, R.M., and TIDWELL, J.H., 2004. *Anesthetics in aquaculture*. Stoneville: Southern Regional Aquaculture Center, No. 3900.

DIVAKARAN, S., OBALDO, L.G. and FORSTER, I.P., 2002. Note on the methods for determination of chromic oxide in shrimp feeds. *Journal of Agricultural and Food Chemistry*, vol. 50, no. 3, pp. 464-467. http://dx.doi.org/10.1021/jf011112s. PMid:11804513.

EL-HAMMADY, A., EL - KASHEIF, M. and IBRAHIM, S., 2007. Synergistic reactions between vitamin E and selenium in diets of hybrid tilapia (*Oncorhynchus mykiss* × *Oreochromis aureus*) and their effect on the growth and liver histological structure. *Egyptian Journal of Aquatic Biology and Fisheries*, vol. 11, no. 1, pp. 53-58. http://dx.doi.org/10.21608/ejabf.2007.1914.

ESSA, A.M., MABROUK, A.H. and ZAKI, A.M., 2004. Growth performance of grass carp, (*Ctenopharyngodon idella*) and hybrid grass carp fingerlings fed on different types of aquatic plants and artificial diet in concrete basins.*Egyptian Journal of Aquatic Research*, vol. 30, pp. 341-348.

FAIZ, H., ZUBERI, A., NAZIR, S., RAUF, M. and YOUNUS, N., 2015. Zinc oxide, zinc sulfate and zinc oxide nanoparticles as source of dietary zinc: comparative effects on growth and hematological indices of juvenile grass carp (*Ctenopharyngodonidella*). *International Journal of Agriculture and Biology*, vol. 17, no. 3, pp. 568-574. http://dx.doi.org/10.17957/IJAB/17.3.14.446.

FEDERICI, G., SHAW, B.J. and HANDY, R.D., 2007. Toxicity of titanium dioxide nanoparticles to rainbow trout, (*Oncorhynchus mykiss*): gill injury, oxidative stress, and other physiological effects. *Aquatic Toxicology (Amsterdam, Netherlands)*, vol. 84, no. 4, pp. 415-430. http://dx.doi.org/10.1016/j.aquatox.2007.07.009. PMid:17727975.

GONZALES-EGUIA, A., FU, C.M., LU, F.Y. and LIEN, T.F., 2009. Effects of nanocopper on copper availability and nutrients digestibility, growth performance and serum traits of piglets. *Livestock Science*, vol. 126, no. 1-3, pp. 122-129. http://dx.doi.org/10.1016/j.livsci.2009.06.009.

HANDY, R.D., CORNELIS, G., FERNANDES, T., TSYUSKO, O., DECHO, A., SABO-ATTWOOD, T., METCALFE, C., STEEVENS, J.A., KLAINE, S.J., KOELMANS, A.A. and HORNE, N., 2012. Ecotoxicity test methods for engineered nanomaterials: practical experiences and recommendations from the bench.*Environmental Toxicology and Chemistry*, vol. 31, no. 1, pp. 15-31. http://dx.doi.org/10.1002/etc.706. PMid:22002667.

HAO, X., LING, Q. and HONG, F., 2014. Effects of dietary selenium on the pathological changes and oxidative stress in loach (*Paramisgurnusdabryanus*).*Fish Physiology and Biochemistry*, vol. 40, no. 5, pp. 1313-1323. http://dx.doi.org/10.1007/s10695-014-9926-7. PMid:24633928.

HASSAN, A.A., HOWAYDA, M.E. and MAHMOUD, H.H., 2013. Effect of zinc oxide nanoparticles on the growth of mycotoxigenic mould.*SCPT*, vol. 1, pp. 66-74.

HUSSAIN, S.M., AFZAL, M., JAVID, A., ASLAM, N., HUSSAIN, M., SHAH, S.Z.H., AZMAT, H. and MUBARIK, M.S., 2015a. Role of phytase supplementation in improving nutrient digestibility in *Labeo rohita* (Hamilton, 1822) fingerlings fed on cottonseed meal based diet.*Indian Journal of Fisheries*, vol. 62, pp. 78-84.

HUSSAIN, S.M., AFZAL, M., JAVID, A., HUSSAIN, A.I., ALI, Q., MUSTAFA, I., CHATHA, S.A.S., SHAH, S.Z.H., HUSSAIN, M. and ULLAH, M.I., 2015b. Efficacy of phytase supplementation on growth performance and mineral digestibility of *Labeo rohita* fingerlings fed on cottonseed meal based diet.*Pakistan Journal of Zoology*, vol. 47, pp. 699-709.

HUSSAIN, S.M., AHMAD, N., JAVID, A., SHAHZAD, M.M., HUSSAIN, M. and ARSALAN, M.Z.U.H., 2018. Effects of phytase and citric acid supplemented corn gluten (30%) meal-based diets on the mineral digestibility of *Cirrhinus mrigala* fingerlings.*Turkish Journal of Fisheries and Aquatic Sciences*, vol. 18, pp. 501-507.

IQBAL, M.Z., WANG, F., HUSSAIN, R., IQBAL, T., ALI, I., RAFIQUE, M.Y. and ALI, S., 2014. Synthesis and characterization of SnO2 nanorods for energy storage applications.*Advanced Science, Engineering and Medicine*, vol. 6, no. 7, pp. 1-6. http://dx.doi.org/10.1166/asem.2014.1563.

KHALAFALLA, M.M.E., EWEEDAHL, N.M., SALEM, M.F. and SALLAM, A.E., 2011. Effects of different levels of selenium supplementation on growth performance, feed utilization, spawning performance and reproduction of the Nile tilapia (*Oreochromis niloticus*). *Egyptian Journal of Aquatic Biology and Fisheries*, vol. 15, pp. 75-91.

KHAN, K.U., ZUBERI, A., NAZIR, S., FERNANDES, J.B.K., JAMIL, Z. and SARWAR, H., 2016. Effects of dietary selenium nanoparticles on physiological and biochemical aspects of juvenile Tor putitora. *Turkish Journal of Zoology*, vol. 40, pp. 704-712. http://dx.doi.org/10.3906/zoo-1510-5.

KHAN, S.H., SARDAR, R. and SIDDIQUE, B., 2006. Influence of enzymes on performance of broilers fed sunflower-corn based diets.*Pakistan Veterinary Journal*, vol. 26, pp. 109-114.

KRISHNAVENI, K., PALANIVELU, K. and VELAVANS, S., 2013. Spiritualizing effect of probiotic and spirulina on growth and biochemical performance in common carp (*Catla catla*).*Int. J. Res. Zool.*, vol. 3, pp. 27-31.

KUMARI, R., GUPTA, S., SINGH, A.R., FEROSEKHAN, S., KOTHARI, D.C., PAL, A.K. and JADHAO, S.B., 2013. Chitosan Nano encapsulated Exogenous Trypsin Biomimics Zymogen-Like Enzyme in Fish Gastrointestinal Tract.*PLoS One*, vol. 8, no. 9, pp. e74743. http://dx.doi.org/10.1371/journal.pone.0074743. PMid:24040333.

KWASEK, K., THORNE-LYMAN, A.L. and PHILLIPS, M., 2020. Can human nutrition be improved through better fish feeding practices? a review paper.*Critical Reviews in Food Science and Nutrition*, vol. 60, no. 22, pp. 3822-3835. http://dx.doi.org/10.1080/10408398.2019.1708698. PMid:31983214.

LE, K.T., FOTEDAR, R. and PARTRIDGE, G., 2013. Selenium and vitamin E interaction in the nutrition of yellowtail kingfish (*Seriolalalandi*): physiological and immune responses. *Aquaculture Nutrition*, vol. 20, no. 3, pp. 303-313. http://dx.doi.org/10.1111/anu.12079.

MUSHTAQ, T., SARWAR, M., AHMAD, G., NISA, M.U. and JAMIL, A., 2006. The influence of exogenous multienzyme preparation and graded levels of digestible lysine in sunflower meal-based diets on the performance of young broiler chicks two weeks

posthatching.*Poultry Science*, vol. 85, no. 12, pp. 2180-2185. http://dx.doi.org/10.1093/ps/85.12.2180. PMid:17135675.

ONUEGBU, U.C., AGGARWAL, A. and SINGH, B.N., 2018. ZnO nanoparticles as feed supplementation on growth performance of cultured African catfish fingerlings.*Journal of Scientific and Industrial Research*, vol. 77, pp. 213-218.

PEAKE, S., 1998. Sodium bicarbonate and clove oil as potential anesthetics for nonsalmonid fishes.*North American Journal of Fisheries Management*, vol. 4, no. 4, pp. 919-924. http://dx.doi.org/10.1577/1548-8675(1998)018<0919:SBACOA>2.0.CO;2.

PELYHE, C. and MIKLOS, M., 2013. Mythes and facts about the effects of nano selenium in farm animals.*European Chemical Bulletin*, vol. 2, pp. 1049-1052.

RAMSDEN, C.S., SMITH, T.J., SHAW, B.J. and HANDY, R.D., 2009. Dietary exposure to titanium dioxide nanoparticles in rainbow trout, (*Oncorhynchus mykiss*): no effect on growth, but subtle biochemical disturbances in the brain.*Ecotoxicology (London, England)*, vol. 18, no. 7, pp. 939-951. http://dx.doi.org/10.1007/s10646-009-0357-7. PMid:19590957.

SHAH, B.R. and MRAZ, J., 2020. Advances in nanotechnology for sustainable aquaculture and fisheries.*Reviews in Aquaculture*, vol. 12, no. 2, pp. 925-942. http://dx.doi.org/10.1111/raq.12356.

SNEDECOR, G.W. and COCHRAN, W.G., 1991. *Statistical methods.8th* ed. USA: Iowa State University.

STEEL, R.G.D., TORRIE, J.H. and DICKEY, D.A., 1996. *Principles and procedures of statistics*.3rd ed. New York: McGraw Hill International Book Company, Inc., pp. 336-352.

TAHIR, M.Z.I., AHMED, I., MATEEN, A., ASHRAF, M., NAQVI, Z.H. and ALI, H., 2008. Studies on partial replacement of fish meal with oilseeds meal in the diet of major carps.*International Journal of Agriculture and Biology*, vol. 10, pp. 455-458.

VIJAYAKUMAR, M.P. and BALAKRISHNAN, V.B., 2014. Evaluating the bioavailability of calcium phosphate nanoparticles as mineral supplement in broiler chicken.*Indian Journal of Science and Technology*, vol. 7, no. 10, pp. 1475-1480. http://dx.doi.org/10.17485/ijst/2014/v7i10.10.

WANG, T., LONG, X., CHENG, Y., LIU, Z. and YAN, S., 2015. A comparison effect of copper nanoparticles versus copper sulphate on juvenile (*Epinephelus coioides*): growth parameters, digestive enzymes, body composition, and histology as biomarkers.*International Journal of Genomics*, vol. 2015, pp. 10. http://dx.doi.org/10.1155/2015/783021.

ZHOU, J., ZHOU, B.O., LENZMEIER, B.A. and ZHOU, J., 2009. Histone deacetylase Rpd3 antagonizes Sir2-dependent silent chromatin propagation.*Nucleic Acids Research*, Vol. 37, no. 11, pp. 3699-3713. https://doi.org/10.1093/nar/gkp233.