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Different Quantity of Selenium Nanoparticles Incorporated Feed on Growth and Biochemical Characteristics of Common Carp *Cyprinus Carpio*

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ABSTRACT

The present study aimed at the effect of different quantity of selenium nanoparticles incorporated feed on growth and biochemical characteristics of common carp *Cyprinus carpio*. Selenium nanoparticles were synthesized and characterized by using UV-Vis, SEM, EDAX and FTIR. Different quantity of selenium nanoparticles such as 10, 20, 30, 40, 50 mg/100 g was prepared by using Fish meal, Groundnut oil cake, Wheat flour and Tapioca flour. Feed utilization parameters and biochemical characteristics in muscle, gill and liver of *Cyprinus carpio* were estimated after 21 days. Absorption spectra of the synthesized nanoparticles were studied using a UV-Vis spectrophotometer and the maximum peak was observed between 240 to 320 nm. SEM image shows that the selenium nanoparticles are spherical and in the micro-scale of 0.37 mm. EDAX spectrum recorded on the selenium nanoparticles shown three peaks located between 0.9 KeV, 1.3 KeV and 2.5 KeV. The FTIR spectrum of selenium nanoparticles was analyzed in the range of 500-5000^{-1} and spectral bands and functional groups are Alcohols, Ketones, Alkynes, Alkyl, Halides and Amines. The final condition factor increased in all the feeds. The survival rate of Common carp is 100% in feed III and IV. Feed consumption and Feed conversion efficiency of common carp was higher in Feed V. Feed conversion ratio was good in Feed VI. The growth and specific growth rate were higher in Feed VI. Assimilation and metabolism were higher in Feed III and VI. Gross growth efficiency and net growth efficiency was higher in Feed VI and IV. Total protein, carbohydrate and lipid in muscle, gill and liver of Common carp were higher in Feed VI

Key words: Selenium Nanoparticles, Growth, Biochemical, Common Carp

INTRODUCTION

Nanotechnology plays an important role in the economy and society shortly, as is being observed in computers, cellular or molecular biology and many other fields. Nanotechnology is about to demonstrate its important role in areas such as medicine, materials and manufacturing, electricity, informatics and electronics [1]. Nanotechnology is an important and evolving scientific resource for developing environmentally friendly, effective methods for the synthesis of nanoscale materials [2]. Nanoparticles are commonly used in various fields such as biomedical, physics, chemistry, and material sciences [3]. Nanoparticles play a vital role in the development of food products against microbial contamination since it serves as a carrier of nutrients, enzymes, food additives and also enhances the physical, chemical and nutritional quality of feed [4]. Copper, gold, zinc, silver and iron are the most commonly used nanoparticles that have broad applications. Among various

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nanoparticles, selenium plays a vital role in the field of nanotechnology. Although much research has been done on metals, studies on metalloids, such as selenium, etc., are scanty [5]. Selenium proved to be an essential mineral required for proper health, immunity and reproductive functions of animals. It has an important role in resistance to disease either through enhancing the immune response, leukocyte function or specific immunity of the animals, selenium is supplied to the human with food. Selenium plays an important role in fish health and immunity. Symptoms of selenium deficiency on fish include low glutathione peroxidase activity, reduced growth, impaired reproduction, anemia, exudative diathesis, muscular dystrophy and increased mortality. Selenium helps in protecting fish body cells and cellular components from oxidative damage through the activation of selenoenzymes and selenoproteins [6]. Selenium is required for the normal growth, development and flesh quality of fish. The work related to the synthesis, characterization and different quantity of selenium nanoparticles incorporated feed on growth and biochemical characteristics of common carp is wanting. Hence the present study was carried out.

EXPERIMENTAL METHODS

1. Synthesis of Selenium Nanoparticles

Synthesis of selenium nanoparticles was carried out using the chemical precipitation method. For the synthesis of selenium nanoparticles, a solution of 100 mM Sodium selenite and 50 mM Ascorbic acid was prepared. The Ascorbic acid was added dropwise to the sodium selenite solution under magnetic stirring at 2500 rpm at room temperature for 30 min. The Y The mixtures were allowed to react with each other in the concentrated form till the color change was observed from colorless to light orange. Then this precipitate was filtered and then collected in a Petri plate. The precipitate was dried at room temperature.

2. Characterization

a) UV-Vis Spectroscopy

The optical property of selenium nanoparticles was determined by a UV-Vis spectrophotometer. Absorption spectra of the synthesized nanoparticles were studied using a UV-VIS spectrophotometer at a wavelength range of 200-800 nm.

b) Scanning electron microscope (SEM)

SEM is a powerful investigating tool that used a focused beam of electrons to produce complex, high magnification images of a sample's surface topography. The morphology of Se NPs was investigated using a scanning electron microscope (SEM) (LEO 1455 VP).

c) Energy dispersive X-ray spectroscopy (EDAX)

A minute drop of nanoparticles solution was cast on aluminium foil and subsequently dried in the air before transferring into the microscope. An energy-dispersive X-ray detection instrument (EDAX)

d) Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) is used to measure the vibration modes of functional groups of molecules and is sensitive to molecular structure, conformation and environment. Therefore, in the current study, it is possible to relate the intensity of the absorption bands to the concentration of the corresponding functional groups. FTIR spectroscopy was analyzed in the range of 4000-400cm^{-1}. The FTIR spectra of synthesized Se nanoparticles were analyzed for knowing the possible functional groups. The measurement was carried out by JASCO (FTIR-6200) spectrum.

3. Collection of Fishes

Fingerlings of *Cyprinus carpio* (12.6 ± 0.02 g) were purchased from Pandian fish farm, Thirumalaikeni,

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Dindigul, Tamil Nadu, India and acclimatized to laboratory conditions for 15 days before the commencement of the experiment. Feeding was done at least one hour before the replacement of water. Water (one-third) was changed frequently to remove the excretory wastes. Feeding was stopped for 24 h before the commencement of the experimental animals more or less in the same metabolic state. During acclimatization, the fish stock was maintained at natural photoperiod and ambient temperature. This ensures sufficient oxygen for the fish and the environment is devoid of any accumulated metabolic wastes. The initial body weight and length of the fish were measured in gram and cm respectively.

4. Feed preparation

The raw materials are selected based on their ability to supply nutrients such as protein, carbohydrate and fat at low cost. Wheat flour, tapioca flour was used as carbohydrate sources. Vegetable oil was used as a lipid source and served as binding agents; supplevite mix was added. The components used for feed preparation was dried, powdered and sieved through a 425-micron sieve. The ingredients were weighed and mix thoroughly with 10-150 ml of distilled water. The mixed feedstuff was put in an autoclave for 15 min at 100˚C and cooled. After cooling, fish oil, supplevite-mix, sodium benzoate and different quantities of selenium nanoparticles such as 10, 20, 30, 40 and 50 mg. The mixture was dried at room temperature and stored in plastic containers (Table 1).

Table 1: Composition of Different Feed Ingredients in Experimental Feeds (g/100gm) of Common carp

Ingredients	Experimental Feeds					
	Feed I (control)	Feed II	Feed III	Feed IV	Feed V	Feed VI
Fish metal	36.2	36.2	36.2	36.2	36.2	36.2
GNOC*	36.2	36.2	36.2	36.2	36.2	36.2
Wheat flour	8.7	8.7	8.7	8.7	8.7	8.7
Tapioca	8.7	8.7	8.7	8.7	8.7	8.7
Fish oil	2	2	2	2	2	2
Sunflower Oil	2	2	2	2	2	2
Supplevite mix	4	4	4	4	4	4
Sodium Chloride	1	1	1	1	1	1
Sodium Benzoate	1	1	1	1	11	1
Selenium Nanoparticles	0	10 mg	20 mg	30 mg	40 mg	50 mg
*GNOC - Groundnut Oil Cake						

5. Growth parameters

Growth parameters such as Condition factor, feed consumption, feed conversion efficiency, feed conversion ratio, growth, specific growth rate, assimilation, metabolism, gross and net growth efficiency were calculated after 21 days. Biochemical characteristics such as total protein, carbohydrate, and lipid [7-9] were also estimated.

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RESULTS AND DISCUSSION

The optical property of selenium nanoparticles was determined by a UV-Vis spectrophotometer. Absorption spectra of the synthesized nanoparticles were studied using a UV-Vis spectrophotometer at a wavelength range of 200-800 nm. The maximum peak was observed between 240 to 320 nm (Figure 1). UV-Vis spectrum is the most basic and important technique for the identification and characterization of nanoparticles [10]. The UV-Visible absorption maxima at 395 nm for nano selenium [11]. A characteristic peak was observed in the UV spectrum at 320 nm confirming the presence of selenium nanoparticles and the peak at 440 nm confirmed the formation of selenium nano biocomposites [12].

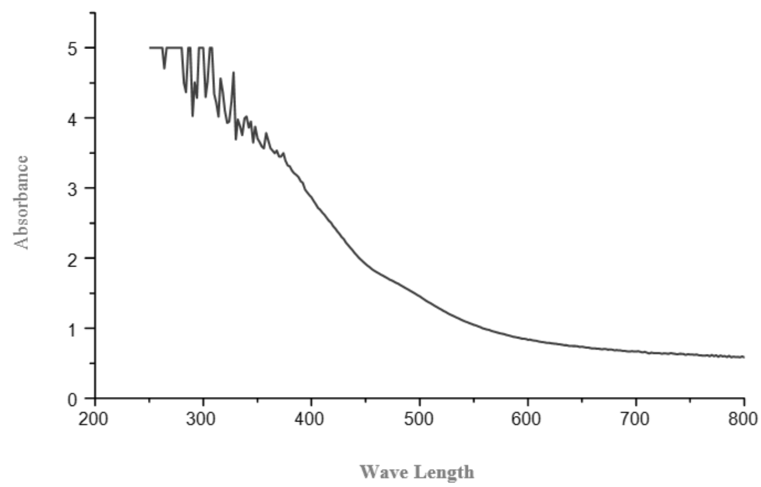

Figure 1: UV-Vis Spectrophotometer Image of Selenium Nanoparticles

SEM image of Selenium is taken for the analysis of the size and shape of SeNPs. A closer look at the highly magnified emission scanning electron microscopy image is useful in suggesting the composition of the nanoparticles as well as to observe the nature of the surface of the nanoparticles. Obtained nanoparticles are spherical and also the microscopic image showed that the micro-scale size for the SeNPs range is about 10.37 nm (Scale 2 µm) (Figure 2). The SEM image of Se nanoparticles greater numbers of the agglomerated spherical particles within the diameter range 45-90 nm and were well distributed with aggregation [13]. Also reported that the morphology of the selenium nanoparticles are spherical with the size of 35.6 ± 7.5 nm [14].

The chemical composition of the synthesized selenium nanoparticles was also confirmed by EDAX. The EDAX spectrum of the nanoparticles is shown in Figure 3. The EDAX of the selenium was analyzed in the range from 0 to 5 Kev. The maximum selenium nanoparticles compounds shown at one peak located in 1.4 kev. The maximum peak located on the spectrum 1.4 kev (83.52%) coming from selenium. The EDAX of the selenium was analyzed in the range from 0 to 5 Kev. Selenium peak at 1.4 and 11.1 keV also confirms the reduction of sodium selenite to selenium nanoparticles [12]. The maximum selenium nanoparticles compounds shown at one peak located in 1.4 Kev. The EDAX profile shows a strong selenium signal along with weak sulfur group peaks [15]. The result indicated that 92.76% (wt.) of the sample had the presence of selenium nanoparticles.

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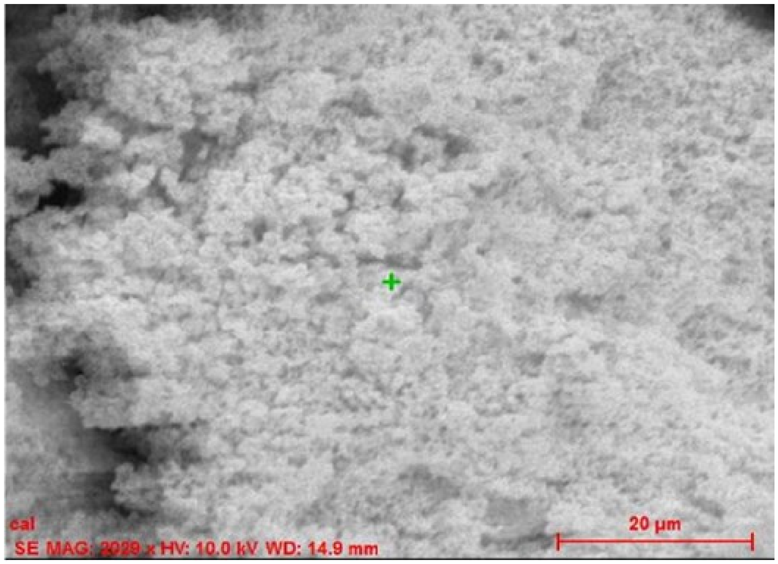

Figure 2: Scanning Electron Microscopic (SEM) Image of Selenium Nanoparticles

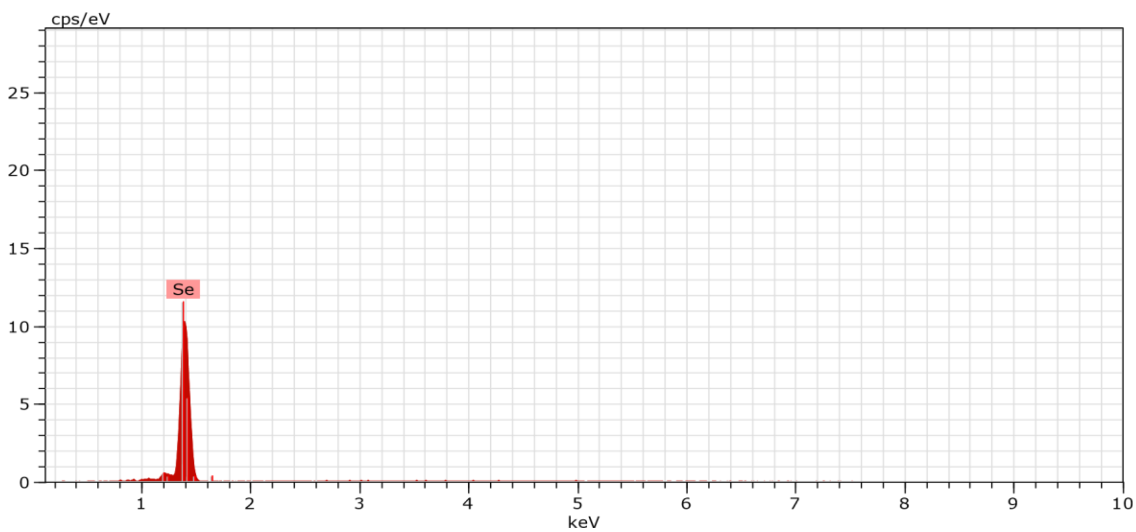

Figure 3: Energy Dispersive X-Ray Spectroscopy (EDAX) of Selenium Nanoparticles

Fourier Transform Infrared Spectroscopy measurements were carried out to identify the possible functional groups responsible for the reduction of selenite into selenium nanoparticles. The peaks obtained were plotted as the % transmitted Y-axis and wavelength nm X-axis. The FTIR spectrum of the selenium was analyzed in the range 4000-500 cm and bands observed at 3992.89, 2999.73, 2928.73, 2999.73, 2319.37, 2049.96, 1632.44, 1481.06, 588.04, and 513.93 which are associated with 0-H stretch, CH3C-H bend, C-F stretch, O=C-O-C (Figure 4). The vibrational and stretching of functional groups at wavenumbers 2,356.2, 1,618.7, 1,284.7, 2,360.1, 1,723.2 and 1,638 cm^{-1} and were correspond to stretching and vibrational bending of C=C, NH2, COOH, CH2 and C=O, thus, indicating the presence of reducing groups responsible for the reduction of nano selenium [16]. The spectrum of nano selenium has vibrational and stretching functions at wave numbers 2919.69, 1630.51, 1380.78 and 1076.08 cm^{-1} corresponding to C-H, C=C, O-H and C-O respectively, suggesting the presence of reducing groups aiding in nano-selenium fabrication [17]. Multiple bands of aromatic (C=C) bonds

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from 1400 to 1200 cm^{-1} having medium stretch vibrations were also observed in selenium nanoparticles [12].

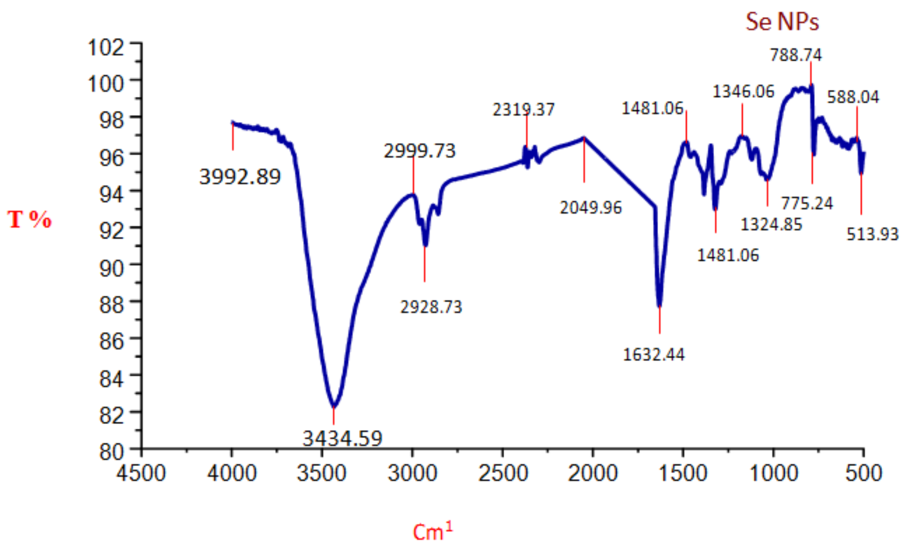

Figure :4 Fourier Transform Infrared Spectroscopy of Selenium Nanoparticles

The condition factor of Common carp *Cyprinus carpio* reared in different feeds were presented in Figure 5. Condition factor (K) of Common carp was estimated for comparative purposes to assess the feed. The final condition factor of common carp was increased in all the feeds supplemented with selenium nanoparticles. Also reported an increase in condition factor of Macrobrachium rosenbergii post-larvae fed with 40 g/ kg^{-1} of iron oxide nanoparticles in the feed [18].

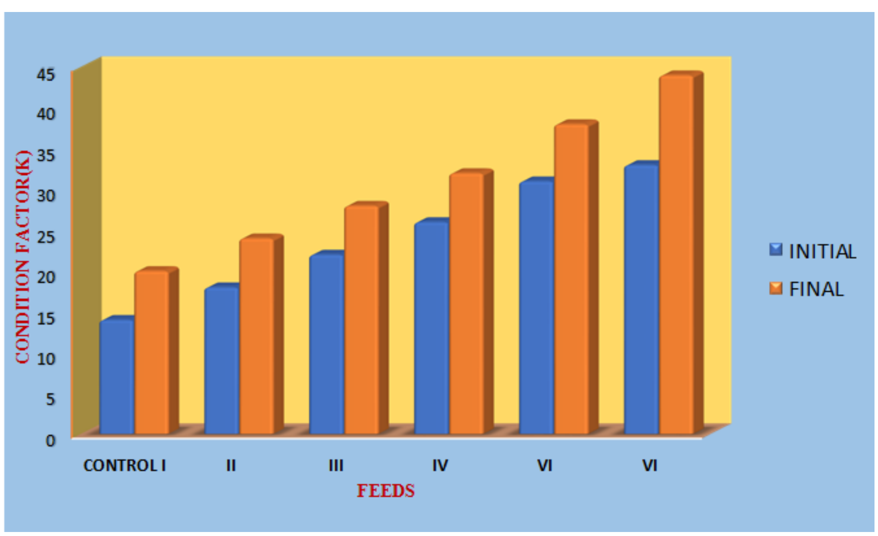

Figure 5: Condition Factor (k) of Common carp

Survival and growth parameters of Common carp grown in different quantity of selenium

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incorporated feed are presented in Table 2. The survival of common carp is 100, 90, 100, 100, 80 and 90% for feed I, II, III, IV, V and VI respectively. The survival of *Cirrhinus mrigala* was 100% in feed containing 0, 5 and 20 mg of zinc oxide nanoparticles, and 80, 90, and 100% were observed in 10, 15 and 25 mg [19]. Feed consumption and feed conversion efficiency of Common carp were higher in feed V (3.26 ± 0.5 and 0.18 ± 0.01) containing 40 and 50 mg/g^{-1} of selenium nanoparticles respectively. An increase in the concentration of zinc oxide nanoparticles with feed consumption and feed conversion in African Catfish fingerlings [20]. The feed conversion ratio was good in Ex. Feed VI (2.15). The feed conversion ratio was higher in control and lower of zinc oxide fed of *Macrobrachium rosenbergii* [21]. The feed conversion ratio was higher in ZnO nanoparticles incorporated feed of juvenile grass carp [22]. Growth and Specific growth rate were higher in Feed VI (50 mg/g^{-1}) when compared to control and significantly increased. Green synthesized zinc oxide nanoparticles incorporated feed improved the growth of *Labeo rohita* [23]. A higher specific growth rate was reported when Mrigal fingerlings fed with a diet containing 15 mg of ZnO-NP per 100 g [19]. Assimilation of Common carp is higher in feed III and metabolism is higher in feed VI. The gross growth efficiency of Common carp is higher in feed V and the net growth efficiency of Common carp is higher in feed V containing 40 mg of selenium nanoparticles.

Table 2: Survival and Growth Parameters of Common carp to the different quantity of Selenium nanoparticles incorporated feeds. Each value is the average (± SD) 10 individuals in triplicate.

Parameters	Experimental Feed					
	Feed I (Control)	Feed II (10 mg)	Feed III (20 mg)	Feed IV (30 mg)	Feed V (40 mg)	Feed VI (50 mg)
Survival Rate (%)	100	90	100	100	80	90
Feed Consumption (g/g live wt/21 days)	1.33 ± 0.1^a	1.5 ± 0.2^b	12.83 ± 0.46^c	2.96 ± 0.46^d	3.1 ± 0.92^e	3.26 ± 0.5^f
Feed Conversion Efficiency	0.05 ± 0.02	0.04 ± 0.05	0.08 ± 0.04	0.12 ± 0.02	0.18 ± 0.01	0.1 ± 0.01
Feed Conversion Ratio	3.35 ± 0.53	4.05 ± 0.32	6.21 ± 0.45	7.05 ± 0.52	3.85 ± 0.71	2.15 ± 0.45
Growth (g/g live wt/21 days)	0.25 ± 0.05^a	0.36 ± 0.08^b	0.48 ± 0.04^c	0.76 ± 0.03^d	0.58 ± 0.05^e	0.68 ± 0.05^f
Specific Growth Rate (%)	1.52 ± 0.12	2.02 ± 0.16	0.53 ± 0.21	0.90 ± 0.10	1.8 ± 0.23	3.2 ± 0.31
Assimilation (A)	1.2 ± 0.16	1.53 ± 0.61	1.6 ± 0.25	1.45 ± 0.34	0.87 ± 0.25	1.2 ± 0.38
Metabolism (M)	1.16 ± 0.20	1.2 ± 0.30	1.18 ± 0.52	1.3 ± 0.12	1.53 ± 0.48	2.3 ± 0.24
Gross Growth Efficiency (%)	24.2 ± 0.75^a	27.2 ± 1.8^b	29.6 ± 2.6^c	30.4 ± 3.5^d	26.1 ± 2.7^e	44.03 ± 1.7^f
Net Growth Efficiency (%)	19.3 ± 2.32^a	26.3 ± 2.07^b	24 ± 1.33^c	18.5 ± 2.54^d	28.6 ± 1.73^e	20.53 ± 1.7^f

Feed consumption	Growth	Gross growth efficiency	Net Growth efficiency
avs b (P>0.05) S	a vs b (P>0.05) S	a vs b (P>0.05) S	a vs b (P>0.05) S
avs c (P>0.05) S	a vs c (P>0.05) S	a vs c (P>0.05) S	a vs c (P>0.05) S
avs d (P>0.05) S	a vs d (P>0.05) S	a vs d (P>0.05) S	a vs d (P>0.05) S

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avs e (P>0.05) S	a vs e (P>0.05) S	a vs e (P>0.05) S	a vs e (P>0.05) S
avs f (P>0.05) S	a vs f (P>0.05) S	a vs f (P>0.05) S	a vs f (P>0.05) S

Total protein, carbohydrate and lipid content of muscle, gill and liver of Common carp gradually increased when the concentration of selenium nanoparticles increased (Table 3). The selenium nanoparticles in the feed increased the protein, carbohydrates and lipid content of muscle, gill and liver on Common carp *Cyprinus carpio* [24]. The addition of diet with nano-Se significantly alter the biochemical parameters in rainbow trout (*Oncorhynchus mykiss*) and also increases the level of protein [25]. The iron oxide nanoparticles altered the biochemical parameters of *Labeo rohita* [26]. Also reported a concentration-based increase and decrease of protein, carbohydrate and lipid [27].

Table 3: Total protein, carbohydrate and lipid in muscle, gill and liver of Common carp

Feed		Protein (mg/g)	Carbohydrate (mg/g)	Lipid (mg/g)
I	Muscle	0.34	1.07	1
	Gill	0.44	0.25	0.76
	Liver	0.04	0.23	36
II	Muscle	0.46	1.22	1.21
	Gill	0.48	1.23	0.48
	Liver	0.07	0.74	0.45
III	Muscle	1.2	1.45	1.35
	Gill	0.52	2.66	1.07
	Liver	0.1	1.05	0.78
IV	Muscle	1.3	2.03	1.97
	Gill	0.67	2.86	1.33
	Liver	0.15	1.24	0.67
V	Muscle	1.5	2.66	2.02
	Gill	1.23	3.95	1.51
	Liver	0.22	1.95	0.98
VI	Muscle	1.9	3.05	2.22
	Gill	1.45	4.23	1.99
	Liver	0.52	2.43	1.09

CONCLUSION

This study successfully demonstrates the acceptable application of selenium nanoparticles and subsequent utilization as nutrient for the growth and biochemical characteristics of Common carp.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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