Research Article

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Incorporation of Nano Selenium in Fish Diet and Assessment of Growth Performance and Biochemical Criteria of *Labeo rohita*

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ABSTRACT

Selenium (Se) is an essential microelement utilized in aqua-feeds for aquatic animals' normal growth, well-being and health. This work was intended to assess the growth performance of *Labeo rohita* (Rohu) resulting from the nano Se-merged diet. Nano Se was synthesized and its physico-chemical characteristics were characterized using UV-VIS, SEM, EDAX, XRD and FTIR for varying quantities of nano Se; Diets - I-0, II- 0.5, III-1, IV-1.5, V-2 and VI- 2.5 mg/ kg were prepared, making use of fish meal (FM), groundnut oil cake (GNOC), wheat flour (WF) and tapioca flour (TF). Feed-utilizing parameters and the biochemical composition of Rohu were evaluated subsequently after 28 days. The UV-Vis Spectroscopy revealed that nano Se was assessed in wavelengths of 200 to 500 nm and exhibited strong absorption at 322 nm. SEM image showed spherical morphology with an average particle diameter of 12.22 mm. EDAX spectrum recorded two signals at 1.5 keV and 11 keV. XRD patterns showed crystalline characteristics of nano Se at 2θ correlators of 23.5º, 29.7º, 41.4º, 43.6º, 45.4º, 51.7º, 55.9º and 61.5º. FTIR spectrum was examined in the range of 4000 – 500 cm^{-1}. Rohu's growth performance and biochemical analysis revealed that the protein, carbohydrate and lipids of gill, liver and muscle of Rohu were highly increased in the case of diet IV containing 1.5 mg/kg nano Se.

Keywords: Nano Se; Rohu; Diet; Growth; Biochemical.

1. INTRODUCTION

One of the most exciting new technologies of the twenty-first century is nanotechnology. The principles of nanoscience can be applied to practical situations through the observation, measurement, manipulation, integration, control, and manufacture of materials at the nanoscale scale (Bayda *et al.* 2020). Nanotechnology focuses on creating a variety of nanoparticles like Se, Cu, Fe, FeO, Zn and ZnO, useful in biological applications, notably in drug and nutrient delivery. Aquaculture, an industry that is expanding quickly, is now a major contributor to the global production of aquatic animals. Intense aquaculture systems have gained popularity as a result of expanding fish demand, which has increased fish stress levels and aggravated the emergence of diseases that have resulted in significant financial losses (Gabriel *et al.* 2022; Kohshahi *et al*. 2019). To support high productivity and variety in fish farming, the use of nanotechnology and adaptation of good fishery management practices are of primary importance (Nemati *et al*. 2019). In aquatic organisms, nanoparticle incorporation in fish diet improves perpetuation, growth, antioxidant levels and immunity (Khan *et al*. 2020). Selenium is an essential trace element for maintaining the entire body functions of the fish including growth, feed efficiency, immune and metabolism of aquatic organisms (Kim and Kang, 2014). When selenium is present in adequate concentrations in fish diets, protecting against oxidative damage (Atencio *et al.* 2009), selenium is required for the function of several significant seleno-proteins, including glutathione peroxidase (GPx) responses of fish (Kumar *et al.* 2018). In a few reports, a selenium-free diet causes malnutrition, and abnormal body activities were observed in fish diversity (Wang *et al.* 2021) The economic significance of Rohu is a key justification for its use as an experimental animal. Moreover, one of India's most notable commercially farmed species is Rohu. According to recent research, feed additives based on nanotechnology have a good effect on aquaculture. This study aims to assess the effect of the nano Se incorporated diet in different quantities on Rohu *Labeo rohita*'s growth and biochemical composition such as protein, carbohydrate and lipid contents of gill, liver and muscle.

2. MATERIALS AND METHODS

2.1 Synthesis of Nano Se

The precipitation technique is used for the synthesis of nano Se. 100 mM of sodium selenite (Na_2SeO_3) was made as a solution in 100 ml of deionized water, and to homogenize, the solution was stirred vigorously for 10 minutes using a magnetic stirrer. 50 ml

of 50 mM ascorbic acid ($C_6 H_8 O_6$) was added dropwise under continuous stirring, to achieve the precipitation of nano Se. The observed starting pH was 6.5. The process was continued until the solution became orange-red color precipitate formation. Then the precipitated solution was allowed to centrifuge at 6000 rpm for 20 minutes. The obtained pellets were washed with 2 ml of distilled water 2 times and continued with 2 ml of ethanol 2 times to remove the impurities. The collected pellets were air-dried at room temperature. Finally, the dried pellets were collected in an air-tight container for further characterization and feed incorporation (Vahdati and Tohidi, 2020).

2.2 Characterization of Nano Selenium

Nano Se was characterized by using a UV-Vis spectrophotometer (Spectra UV-VIS Double Beam DUV 3500), SEM (LEO 1455 VP), EDAX (HORIBA 8121-H), XRD (SHIMADZU Model XRD 6000), and FT-IR analysis (JASCO (FTIR- 6200) Spectra) (Senthamarai and Rajan, 2023).

2.3 Collection and Acclimation of Rohu

The Rohu fingerlings (2±0.5 g) were collected and taken away from the Palar fish seed farm in Palani, Tamil Nadu, India to the test site in an oxygen-rich water receptacle. The collected fish were fed with control feed for 2 weeks at room temperature. Ten fishes were kept in triplicate to assess Rohu's growth and biochemical characteristics.

2.4 Choice of Diet Constituents and Empirical Diet Provision

The micro-Kjeldhal method was utilized to determine the protein content; the basic materials were chosen based on their capability to provide nutrients. Protein sources included groundnut oil cake and fish meal; as the provenance of carbohydrates, tapioca flour and wheat flour were utilized; fish oil (FO), sunflower oil (SFO), suppletive mix, sodium chloride (NaCl), sodium benzoate ($C_7H_5NaO_2$) and various quantities of nano Se such as 0, 0.5, 1, 1.5, 2, 2.5 mg/kg, for diet I-0 as control, diet II, diet III, diet IV, diet V and diet VI respectively, were mixed using sterile deionized water (Table 1). The ingredients were thoroughly mixed, and the mixed feed was sterilized for 15 minutes at 100 °C. The feed was allowed to cool, and then extruded using a pelletizer. The pellets were allowed to dry at room temperature. The prepared diet was stored in an air-tight box before being used to prevent contamination (Rajan and Pavithra, 2023).

Table 1. Combination of differential constituents in the test diet (g/100 gm) of Rohu

Constituents	Test Diets					
	I (Control)	II	III	IV	V	VI
Fish Meal	34.375	34.375	34.375	34.375	34.375	34.375
GNOC*	34.375	34.375	34.375	34.375	34.375	34.375
Wheat Flour	14.125	14.125	14.125	14.125	14.125	14.125
Tapioca Flour	14.125	14.125	14.125	14.125	14.125	14.125
Fish Oil	2	2	2	2	2	2
Sunflower oil	2	2	2	2	2	2
Supplevite-Mix	2	2	2	2	2	2
Sodium Chloride	2	2	2	2	2	2
SodiumeBenzoate	2	2	2	2	2	2
Nano Se (mg/kg)	0	0.5	1	1.5	2	2.5

*GNOC-Groundnut Oil Cake

2.5 Experimental Design

Rohu (2 ± 0.5g) was chosen for this study, and the fish were introduced in an 18-litre glass tank. Ten healthy fishes were acquainted in each tank and triplicates were maintained for each treatment. The feeding frequency was twice daily (8 to 9 am and 2 to 3 pm). The unfed was removed after one hour from the feeding time, without interrupting the fish and dried to consistent heaviness. Before changing the water, the fish faeces were collected daily and dried at 70 °C, and experimental water was renewed at approximately 70%. The experiment was terminated on the 28th day.

2.6 Analysis of Growth Parameters of Rohu

Feed utilization parameters corresponding to condition factor (CF), feed consumption (FC), feed conversion efficiency (FCE), feed conversion ratio (FCR), growth, assimilation, metabolism, gross growth

efficiency (GGE), net growth efficiency (NGE) and biochemical composition and protein, carbohydrate and lipid examination of Rohu were analyzed from gill, muscle and liver after 28 days study. The following formulae were used to calculate the growth parameters of Rohu (Soundhariya and Rajan 2021). i) FC=Feed given (g) – unfed (g)/number of fishes x 100; ii) FCE=Final weight of fish (g) – Initial weight of fish (g)/feed intake; iii) FCR= FCR = Total dry feed intake (g)/Wet weight gain (g); iv) Growth= (Final weight (g) – Initial weight (g)/Initial weight (g); v) Assimilation = Feed consumption (g) - Faecal matter (g); vi) Metabolism= Assimilation (g) – Growth rate (g); vii) Gross growth efficiency= Growth (g)/Feed consumption (g) x 100; viii) Net growth efficiency = Growth/Assimilation x 100; ix) Condition factor = Total weight of fish (g)/Total length of fish $(cm)^3$ x 100.

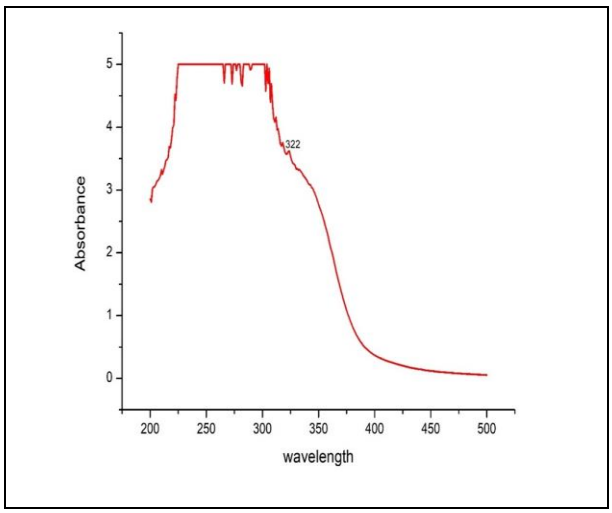

Fig. 1: UV-Visible Spectroscopy image of Nano Se

2.7 Biochemical Criteria of Rohu

2.7.1 Total Protein Estimation

To estimate the total protein, Lowry's method was followed to evaluate the content from the gill, liver and muscle of Rohu (Lowry *et al.* 1951). Bovine serum albumin was used as the standard to determine the protein content of the liver and muscle. A predetermined amount of tissue was mixed with 5% trichloroacetic acid and centrifuged for five minutes at 4000 rpm to obtain a transparent supernatant. Tissue protein was estimated by dissolving the precipitate in a solution of 1% sodium hydroxide. The protein in the sample undergoes a biuret reaction and phosphomolybdic phosphor-tungstic component reduction when it combines with folin phenol reagent, giving rise to a blue colour. Using the reagent blank as a reference, the developed colour was measured in the colorimeter at 500 nm. mg/100 mg was used to express the results.

2.7.2 Total Carbohydrate Estimation

When the anthrone reagent reacts with the hydrolyzed monosaccharides in the sample, a complex and coloured product is formed. The Anthrone technique was used to estimate the amount of carbohydrates (Jermyn 1975). 2 ml of trichloroacetic acid was used to homogenize 100 mg of tissue, and the mixture was centrifuged at 4000 rpm for ten minutes. 4 ml of anthrone reagent was added to half a ml of the supernatant, and the mixture was heated to for 15 minutes. Following the cooling of the test tubes, a photoelectric colorimeter was used to measure the reddish-brown color that had evolved at 630 nm. The mg/100mg expression for the findings was used.

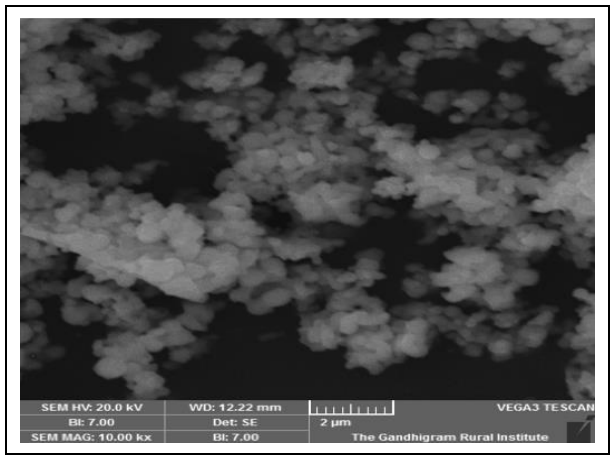

Fig. 2: SEM image of Nano Se

2.7.8 Total Lipid Estimation

The tissue's total lipid content was calculated using Folch (Low and Ng 1975). The tissue was dried in the spaces between the filter paper folds. A weighted amount of gill, liver and muscle were homogenized in a chloroform-methanol mixture after being cleaned with cold, saline ice. For 2 days, the filtered homogenous was kept in its original state. The homogenized extract, after two days, develops two layers. Transferring the lower phase's lipid content to a beaker that had been previously weighed allowed the extracts from the upper phase's chloroform-methanol mixture to evaporate under vacuum at ambient temperature. The lipid extract was then dissolved in a mixer of methanol and chloroform, and aliquots were obtained for quantification.

2.8 Statistical Analysis

Data of growth parameters were analyzed using IBM SPSS software 17. One-way ANOVA was used to determine whether significant or not between the treatments. The significant differences were considered at $P<0.05$.

3. RESULTS AND DISCUSSION

3.1 Characterization of Nano Se

The primary characterization of nano Se using a UV-Vis Spectrophotometer was analyzed in a wavelength range within 200 to 500 nm, and a sturdy fixation band was observed at 322 nm, as shown in Fig. 1. UV–Vis spectrum is the much basic and essential approach for the identification and characterization of nanomaterials. A similar strong absorbance peak between 320 and 550 nm, with λ^{max} of 390 nm, was also reported by Malhotra *et al.* (2014). Prasad *et al.* (2013) reported the UV-visible absorption maxima at 395 nm for nano Se. UV-Vis spectra of Se NPs synthesized from propolis-ethanol extract's strong absorption peak were shown between 250-280 nm with λ^{max} at 265 nm, verifying the presence of selenium in the sample (Kohshahi *et al.* 2019). PUP-Se NPs had a UV-visible absorption peak at 270 nm, while Se NPs showed λ^{max} at 560 nm (Gao *et al.* 2020). SEM exhibited that the nano Se were found in clusters because of the adherent nature of spherical emergence as shown in Fig. 2. It can be seen that selenium nanoparticles were in the form of agglomerates; in addition, SEM depicted the spherical structure of the nano Se with an average size of 35.6 ± 7.5 nm (Shubharani *et al.* 2019). Baskar *et al.* (2019) have synthesized selenium nanoparticles with uniform and smooth surfaces having an average particle size of 20–30 nm. Pavithra *et al.* (2021) reported the morphological features of the Se NPs using SEM. The presence of selenium element was acknowledged by the EDAX analytical technique. The EDAX spectrum evidenced on the nano Se is shown at two signals between 0.1 and 12 keV. These dual peaks indicating the purity of the nano Se were presented on the spectrum at 1.5 and 11 keV. EDAX spectrum of selenium nanoparticles was recorded, and there is only one prominent selenium peak on the spectrum with no other material peaks (Fig. 3). Three peaks were reported by Shubharani *et al.* (2019) from the EDAX: a sturdy signal from the Se atom was 50.79%, together with the O atom was 35.55% and the C atom was 13.66%. Shahabadi *et al.* (2021) reported the EDAX analysis of selenium nanoparticles in which the surface represented a strong band of Se, C, O and N atoms. The XRD patterns obtained the main peaks characteristic of crystalline selenium at 2 θ values of 23.6°, 29.42° and 43.32°. The XRD peaks clearly showed the existence of crystalline Selenium (Fig. 4). Shar *et al.* (2019) reported the selenium peaks centred at 2θ of 23.5°, 29.7°, 41.4°, 43.6°, 45.4°, 51.7°, 55.9° and 61.5° corresponded to the crystal planes of (100), (101), (110), (102), (111), (201), (112) and (202) of JCPDS card No. 06-362. The FTIR spectrum of nano Se was analyzed in the range of 4000-500 cm^{-1}. The FT-IR examination was done to identify the functional groups of active components based on the peak assessment of infrared radiation. In a report by Kumar *et al.* (2018) selenium emergence was proved at 3375.23, 2996.23, 2919.122, 2862.69, 1971.15, 1634.48, 1480.25, 1389.02, 1241.37 and 714.73 cm^{-1} bands have alcohols, alkane, amine salt, aldehyde, allene, conjugated acid, sulfonyl chloride, ether, ester and halo compound (Fig. 5). The emergence of nano Se by ascorbic acid and identified components participating in the fabrication of it was in the range of 4000-400 cm^{-1}.

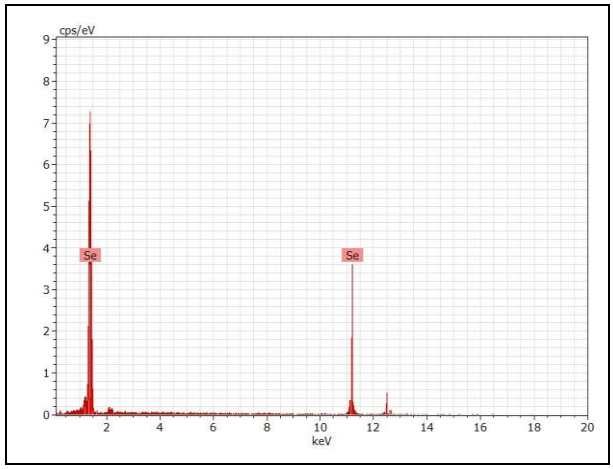

Fig. 3: EDAX image of Nano Se

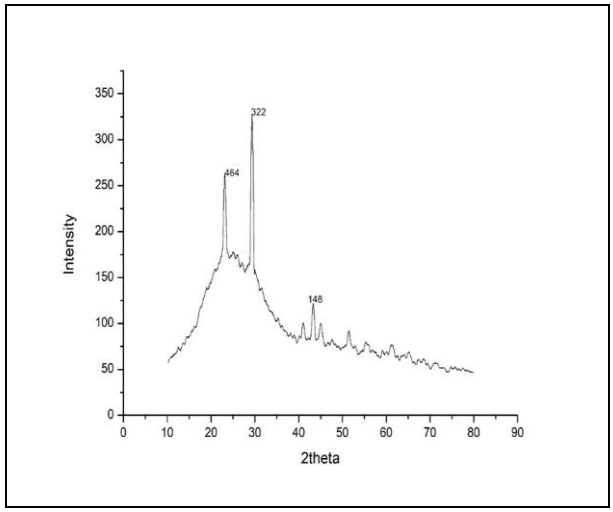

Fig. 4: XRD image of Nano Se

3.2 Feed Utilization Parameters of Rohu

The differential feed usage and growth parameters are listed in Table 2. The statistical analysis of growth parameters data was made using one-way analysis of variance (ANOVA), using SPSS 17 software, and data was represented in Table 3. The condition factor (K) of Rohu studied in differential diets (I, II, III, IV, V and VI) is represented in Table 4. The final condition factor is considerably increased in all diets (Table 4). Similar reports by Moges *et al.* (2022) explained the condition factor (K) value was observed in Nile tilapia, resulting in a 1 mg/kg of Se NPs diet showing high

values. An accrual in condition factor was reported with iron oxide nanoparticles incorporated in the feed of *Macrobrachium rosenbergii* post-larvae fed with 40 g/kg (Muralisankar *et al.* 2016), common carp fed with FeO NPs (Nemati 2019). The feed consumption of Rohu resulted in a higher value of 11.63 ± 1.7 g in diet IV (1.5 mg/kg) than other diet treatments. In similar reports of Sheikh *et al.* (2011), Nile tilapia's feed consumption was significantly increased by 2 mg/kg Se NPs compared to other treatments. A report by Ghaniem *et al.* (2022) stated that SeNPs incorporated at 0.7 mg/kg in the fish diet have significantly increased the fish growth, performance and overall health of Nile tilapia. The feed conversion efficiency of Rohu reared in diet I is 0.513 ±0.04. The feed conversion efficiency in diets II, III, IV, V and VI were 0.376±0.32, 0.378±0.25, 0.48±0.09, 0.413±0.03 and 0.406±0.03, respectively. The FCR ratio of Rohu reared in the feed I is 0.032±0.002, followed by diets II, III, IV, V and VI with 0.017±0.016, 0.018±0.32, 0.041±0.08, 0.044±0.03 and 0.035±0.004, respectively. The growth was higher in feed IV (3.67±0.351) containing 1.5 mg of nano Se. A report by Bisht *et al.* (2022) showed that the growth rates of both control and treatment fish diets were within the normal range for Rohu raised under pond culture, and supplementary Zn and Se had higher growth rates in the treated groups (SGR) than the control group. The assimilation, metabolism, GGE and NGE of Rohu were higher in diet IV (1.5 mg/kg nano Se) and diet V (2 mg/kg nano Se) respectively. A report by Rathore *et al.* (2011) observed 1 mg/kg of Se NPs significantly increased the growth of fish Nile tilapia. The statistical data was analyzed by ANOVA (one-way). Results are represented in Table 3.

Table 2. Feed utilization and Growth parameters of Rohu concerning different quantities of nano Se. Each value is the average (± SD) performance of 10 fish in triplicates raised for 28 days

Parameters	Test diets					
	I	II	III	IV	V	VI
FC*(g)	6.3 ± 1.15	8.9 ± 1.32	9.3 ± 2.06	11.63± 1.7	11.26±0.9	10.2±0.6
FCE*	0.513±0.04	0.376±0.32	0.378±0.25	0.413±0.09	0.48±0.03	0.4±0.03
FCR*	0.032±0.002	0.017±0.016	0.018±0.32	0.041±0.08	0.044±0.03	0.035±0.004
Growth	2.57±0.071	2.286±1.82	2.73±1.94	3.67±0.351	3.567±0.43	2.87±0.288
Assimilation	5.42±1.2	4.56±0.65	7.75±1.37	10.69±1.84	10.20±0.91	8.84±0.69
Metabolism	2.85±1.25	2.28±1.85	5.02±0.57	7.31±2.05	6.93±0.72	2.59±0.79
GGE*	0.419±0.09	0.203±0.16	0.271±0.18	0.295±0.063	0.315±0.021	0.235±0.014
NGE*	0.290±0.08	0.282±0.41	0.286±0.18	0.34±0.07	0.301±0.03	0.278±0.08

The values are represented as mean ± SD.

*FC-Feed consumption; FCE-Feed conversion Efficiency; FCR- Feed conversion Ratio; GGE- Gross Growth Efficiency; NGE- Net Growth Efficiency

Table 3. One-way ANOVA of Growth parameters of Rohu

Parameters	Source of variation	Sum of square	DF	Mean square	F	Sig
Feed Consumption (FC)	Betwist cluster	77.327	5	15.465	8.078	0.002
	Inside cluster	22.973	12	1.914		S
	Total	100.300	17			
Growth	Betwist cluster	17.691	13	1.361	5.921	0.050
	Inside cluster	0.919	4	0.230		
	Total	18.610	17			S
Gross growth efficiency (GGF)	Betwist cluster	0.225	13	0.017	10.205	0.019
	Inside cluster	0.007	4	0.002		S
	Total	0.232	17			
Net growth efficiency (NGE)	Betwist cluster	0.338	13	0.026	0.953	0.581
	Inside Groups	0.109	4	0.027		NS
	Total	0.447	17			

Significant level is P<0.05

Table 4: Condition Factor (K) of Rohu

Diets	Initial	Final
I (Control)	1.008 ± 0.157	1.08 ± 0.122
II	0.883 ± 0.065	0.946 ± 0.023
III	0.87 ± 0.133	1.121 ± 0.164
IV	0.646 ± 0.118	1.06 ± 0.033
V	0.56 ± 0.129	0.939 ± 0.045
VI	0.54 ± 0.151	0.99 ± 0.218

3.3 Biochemical Criteria

Biochemical parameters such as total protein, carbohydrate and lipid content (mg/g) in Rohu's muscle, gill and liver are higher in diet IV (1.5 mg/kg of nano Se), as presented in Table 5. Increased protein, carbohydrates and lipid content of muscle, gill and liver Pavithra *et al.* (2021) reported in crucian carp, Rajan *et al.* (2023) reported in *Oreochromis mossambicus* and *Labeo rohita* were treated with selenium, silver and iron oxide nanoparticles. Ashouri *et al.* (2015) reported that the muscle composition of fish *Cyprinus carpio* supplemented with a diet of 2 mg/kg nano Se has significantly increased. Saffari *et al.* (2017) reported that 0.7 mg/kg of nano Se increased the muscle composition of *Cyprinus carpio* than organic and inorganic Se in the fish diet.

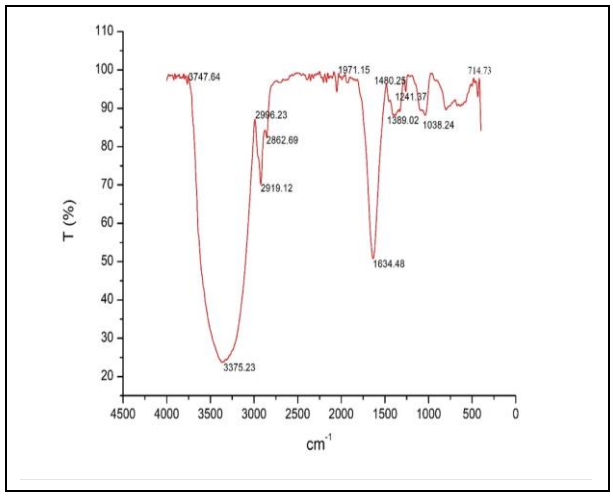

Fig. 5: FT-IR image of Nano Se

Table 5. Total Protein, carbohydrate and lipid (mg/g) in gill, muscle and liver of Rohu

Diets	Parameters*	Protein	Carbohydrate	Lipid
I(control)	G	0.626 ± 0.11	0.065 ± 0.001	0.012 ± 0.1
	M	0.17 ± 0.01	0.081 ± 0.33	0.034 ± 0.03
	L	0.014 ± 0.002	0.008 ± 0.006	0.001 ± 0.020
II	G	0.556 ± 0.020	0.054± 0.003	0.018 ± 0.03
	M	0.84 ± 0.02	0.064 ± 0.016	0.036 ± 0.02
	L	0.016 ± 0.012	0.004 ± 0.003	0.003 ± 0.030
III	G	0.436 ± 0.015	0.084 ± 0.08	0.017 ± 0.002
	M	0.853 ± 0.05	0.122 ± 0.018	0.045 ± 0.013
	L	0.030 ± 0.006	0.004 ± 0.002	0.006 ± 0.013
IV	G	0.87 ± 0.04	0.191 ± 0.005	0.032 ± 0.012
	M	0.976 ± 0.01	0.193 ± 0.025	0.086 ± 0.032
	L	0.036 ± 0.001	0.069 ± 0.002	0.092 ± 0.042
V	G	0.803 ± 0.20	0.128 ± 0.019	0.026 ± 0.02
	M	0.93 ± 0.03	0.143 ± 0.019	0.075 ± 0.03
	L	0.038 ± 0.001	0.056 ± 0.081	0.073 ± 0.032
VI	G	0.79 ± 0.01	0.09 ± 0.004	0.015 ± 0.01
	M	0.723 ± 0.106	0.083 ± 0.006	0.043 ± 0.05
	L	0.027 ± 0.004	0.063 ± 0.006	0.086 ± 0.010

The values are represented as mean ± SD. *G-Gill; M-Muscle; L-Liver.

4. CONCLUSION

From the investigation done, it was evident that a fish diet containing 1.5 mg/kg of nano Se is the recommended level for Rohu's effective growth and biochemical criteria.

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CONFLICTS OF INTEREST

The authors declare that they have no known competing financial interest or personal relationship that could have appeared to influence the work reported in this paper.

ETHICAL CLEARANCE

The experimental design and use of animals for research was carried out as per India's existing Animal welfare law. Treatment and handling of fish for this study were performed as per the supervision and guidance given by the Institutional Ethical Committee for Research on Human and Animal Subjects (IECRHAS), The Gandhigram Rural Institute (Deemed to be University), Gandhigram, Dindigul, Tamilnadu, India.

AUTHOR CONTRIBUTIONS

M. Dayana Senthamarai - Investigation; Writing - Review & Editing; Software.
M. Reka - Data Curation; Formal Analysis; Writing-Original Draft.
M. R. Rajan - Supervision; Validation.

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