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Dietary Supplementation of Zinc Oxide Nanoparticles on Growth, Haematological, and Biochemical Parameters of Koi Carp Cyprinus carpio var koi

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Abstract

The present study aimed at the dietary supplementation of zinc oxide nanoparticles on growth, haematological and biochemical parameters of Koi carp. The zinc oxide nanoparticles were synthesized and characterized using UV-VIS, SEM, EDAX, and FTIR. Six feeds were prepared with different quantities of synthesized zinc oxide nanoparticles (F1 - Control, F2 -20 mg, F3 – 40mg, F4 – 60mg, F5-80mg and F6 – 100mg) and feed ingredients are fish meal, groundnut oilcake, wheat flour, and tapioca flour. Feed utilization, haematological, and biochemical parameters of koi carp were estimated after 21 days. The UV-visible absorption spectra demonstrate that ZnO nanoparticles exhibit strong adsorption at 370nm.SEM image shows that the zinc oxide nanoparticles were observed at the wavelength range from 10.81 mm (scale bar 2μm), 10.8mm (scale bar 10μm). EDAX spectrum recorded on the zinc oxide nanoparticles is shown as two peaks were located on the spectrum between 1.00 KeV and 8.62 KeV. The feed consumption of Koi carp was higher in feed V. The feed conversion efficiency of Koi carp was higher in feed VI. Growth, percentage growth, and assimilation were higher in feed VI. Metabolism of Koi carp was higher in feed III. Haematological parameters of Koi carp were gradually decreased from feed I (control) to feed VI. Total protein, carbohydrate and lipid of muscle, liver and gill of Koi carp was higher in feed V.

Keywords: Dietary; Supplementation; ZnO NPs; Growth; Haematology; Biochemical; Koi carp

Introduction

Nanoparticles are extensively used in various fields such as industrial, biomedical, pharmaceuticals, agricultural, energy, environmental and material applications. Nanoparticles possess unique properties such as large surface area and consequently greater reactivity than macro-sized particles. Nanoparticles play a vital role in the production of food products against microbial contamination, enzymes, food additives and improve the physical, chemical and nutritional quality of feed (Erkan Can et al., 2011) [1]. Nanoparticles such as Se, Cu, Fe, FeO, Zn and ZnO play a vital role in aquaculture operations. The dietary supplementation of nanoparticles produces better survival, growth, antioxidant levels and immunity in aquatic organisms including fish. As the main component of various enzyme systems, zinc takes part in the body's metabolism and plays a crucial role in proteins and nucleic acid synthesis, haematopoiesis, and neurogenesis. Nano-ZnO, with a small particle size, makes zinc more easily to be absorbed by the body. The presence of zinc oxide in fish feed as nano form improved feed palatability thereby causing fish to take more feed. It would cause higher stimulation of synthesis of DNA, RNA and protein leading increase in the body cells of fish. Supplementation of different concentrations of zinc oxide nanoparticles in basal feed greatly improves the weight and biochemical composition of fish (Onuegbu,2018) [2]. Zinc oxide nanoparticle plays a vital role in lipid, protein, carbohydrate and nucleic acid metabolism in fishes (Lall, 2002) [3]. The work-related dietary supplementation of zinc oxide nanoparticles on growth, haematological, and biochemical characteristics of Koi carp is totally wanting. Hence the present study was carried out.

Methods

Materials

For the present work Koi carp fingerlings were collected from Arjun Fish farm Madurai, Tamil Nadu, India and transported to the laboratory in polythene bags filled with oxygenated water. Fishes were acclimated in a cement tank for 15 days at 28°C. During acclimation, fishes were fed with trainee feed containing fish meal, groundnut oil cake, wheat flour and rice brain in the form of dry pellets.

Synthesis of Zinc oxide nanoparticles

The co-precipitation method was used for the synthesis of zinc oxide nanoparticles. For this study, 0.5 M zinc acetate and sodium hydroxide were dissolved separately in distilled water. Precipitation was achieved by adding NaOH. The precipitating process was continued till milky white colour precipitate was obtained. The solution was incubated in a hot air oven at 100 °C for 2 hours. It was centrifuged at 2000 rpm for 10 minutes the centrifuging process continued with distilled water and ethanol in trace volume. The pellet was dried and calcinated, in a hot air oven at 100 °C for 10 minutes. Finally, zinc oxide nanoparticles were formed.

Characterization of Zinc oxide nanoparticles

The synthesized nanoparticles were characterized by UV-Vis Spectrophotometer (JASCO-V-530), Scanning Electron Microscope (SEM)(LEO 1455 VP), Energy Dispersive X-ray detection instrument (EDAX)(HORIBA 8121-H), Fourier Transform Infrared Spectroscopy JASCO(FTIR-6200), and X-Ray Diffraction(SHIMADZU Model XRD-6000).

Experimental Feed preparation

The raw materials are selected on their ability to supply nutrients. Fish meal and groundnut cake were used as protein sources; wheat flour and tapioca flour were used as carbohydrates sources; vegetable oil was used as binding agents; supplevite mix was also added. The components used for feed preparation was dried, powdered and sieved through a 425-micron sieve. After knowing the protein content of major ingredients by Micro-Kjeldahl method the ingredients were weighed and mixed thoroughly with 130-150 ml of distilled water. The mixed feedstuff was put in the autoclave for 15 minutes at 100 °C and cooled. After cooling, fish oil, sunflower oil, supplevite mix, sodium chloride, sodium benzoate and different quality Zinc Oxide nanoparticles (0,20,40,60,80,100 mg/g) were mixed with feed and it was extruded with the help of a pelletizer. The pellets were dried at room temperature. The formulated feed was kept in an airtight container at 0 °C until used to prevent contamination(Table 1).

Methods

Experimental Design for Growth studies

For the present study, koi carp fingerlings (1.5±0.05g) were selected and the fishes were introduced in the glass tank having a capacity of 18 litres. Five fishes were introduced in each trough. For each treatment triplicates were maintained. During rearing the fishes were fed on an ad-libitum diet of the prepared feed twice a day for 1 hour each from 9-10 am and 3-4 pm. The unfed were collected after one hour of feeding without disturbing the fish. The unfed was dried to constant weight. The faecal matter was collected daily before changing the water least disturbance to the fishes and dried at 95 °C. Approximately 70% of water in the tank was replaced with tap water. The experiment was terminated on the 21st day. On the 21st-day growth parameters were calculated. For haematological parameters, blood samples were collected from the cordial vein on the right side of the fish and blood parameters such as Red Blood Corpuscle, haemoglobin, WBC, Haematocrit and Platelets were estimated. Biochemical characteristics such as protein, carbohydrate, and lipid (Lowry et al., 1951, Carrol et al., 1956 & Barnes and Blackstock) [4,5,6] in the muscle, liver and gill of Koi carp was estimated.

Fish used in the present research was in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals [CPCSEA, Ministry of Environment & Forests (Animal Division), Government of India] on the care and use of animals in scientific research and also approved by the Institutional Ethical Committee for Research on Human and Animal Subject (IECRHAS) from - The Gandhigram Rural Institute- Deemed to be University, Govt. of India, Gandhigram, Tamil Nadu, India.

The experimental results are presented in the form of tables using Microsoft Excel (Version 2007) and graphs using Origin (Version 8.0). Mean, Standard deviation, Standard Error and T-test was also calculated with the help of the same tool, One-way ANOVA was used for the analysis using Duncan multiple ranges tests (Duncan, 2012 and Obe, 2014). The data was input manually and computed. The output results obtained from the software indicate whether the difference is between the treatments and days. Sum of square variations (SS), Degree of freedom (dF), Variability of sample means (MS), Critical probability value (F), and Probability (Prob.) was also obtained.

INGREDIENTS	Experimental Feeds					
	Feed I (control)	Feed II	Feed III	Feed IV	Feed V	Feed VI
Fish meal	33.75	33.75	33.75	33.75	33.75	33.75
GNOC	33.75	33.75	33.75	33.75	33.75	33.75
Wheat flour	11.25	11.25	11.25	11.25	11.25	11.25
Tapioca	11.25	11.25	11.25	11.25	11.25	11.25
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
Zinc Oxide Nanoparticles	0	20mg	40mg	60mg	80mg	100mg

GNOC – Ground nut oil cake

Table 1: Composition of different components in Experimental feed (g/100gm) of Koi carp

Results and Discussion

Characterization of ZnO Nanoparticles

UV-Visible absorption spectroscopy is a widely used technique to examine the optical properties of the nanosized particle. The absorbance spectra of zinc oxide nanoparticles exhibit a strong absorption band at 370 nm (Figure 1). The same absorption peak of 370nm was reported in chemically synthesized ZnO nanopowder (Jamdagni et al 2018) [7]. Also reported that the ZnO NPs have a strong absorption maximum at a wavelength of 362nm (Ahmadi Shadmehri et al 2019) [8]. Scanning electron microscopy shows that nanoparticles formed clusters because of the adhesive nature of flower-shaped appearance (Figure 2). A similar flower-shaped appearance of ZnO NPs was also reported (Rajan and Rohini,2021) [9]. The SEM image of ZnO NPs synthesized using zinc acetate is spherical in shape with more aggregation (Jeyabharathi et al 2017) [10]. The elemental composition of ZnO Nps was determined by using EDAX analysis and zinc (Zn) is higher when compared to oxygen. EDAX spectrum recorded on the zinc oxide nanoparticles is shown at two peaks located between 0.40 KeV and 9.0 KeV (Figure 3). Sadhan Kumar Chaudhuri and Lalit Malodia (2017) [11] reported that EDAX analysis was carried out to determine the elemental composition and stereochemistry of the synthesized zinc oxide nanoparticles. The FTIR spectrum of zinc oxide nanoparticles were analysed in the range of 500-4000cm^{-1}. The FT-IR measurement was carried out for identifying the functional groups of bioactive components based on the peak value in the region infrared radiation. Zinc oxide formation was confirmed 3996.74, 3446.17, 2934.16, 2886.66, 2333.44. 1633.41, 1510.95, 1401.99, 1185.04, 827.31, 723.17 and 519.72 cm^{-1} bands have alcohol, amine salt, α, β unsaturated ketene, nitro compound, carboxylic acid, ester, alkene, aromatic compound and halogen compound (Figure 4). The Zn-O bond is assigned to the stretching modes at 400-700 cm-1 for pure ZnO, which is shifted in their position at 691.58, 692.20, and 676.56 cm-1 at the heating times for 3, 4, and 5 h, respectively were reported by Purwaningsih (2016) [12].

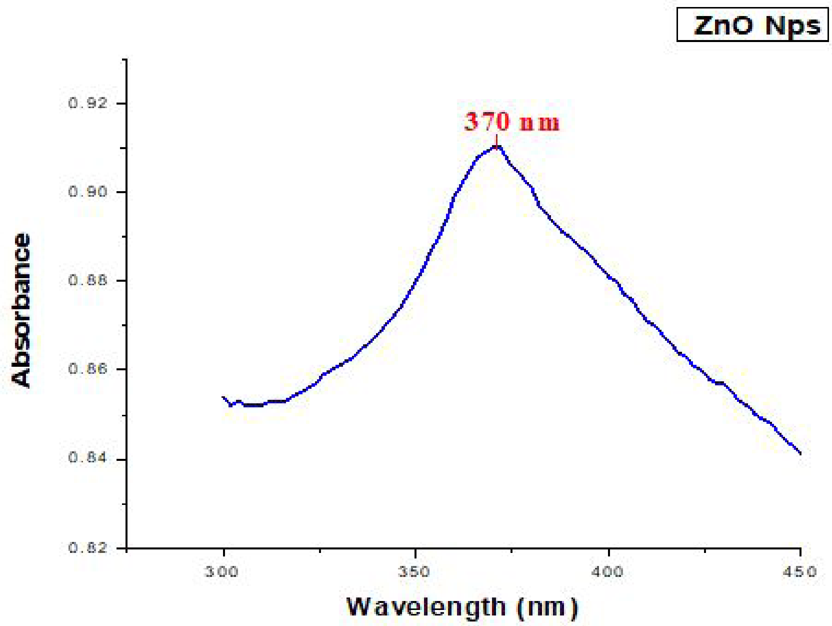

Figure 1: UV-Visible Spectroscopy Analysis of Zinc oxide nanoparticles

(2a 2µm) (2b 10µm)

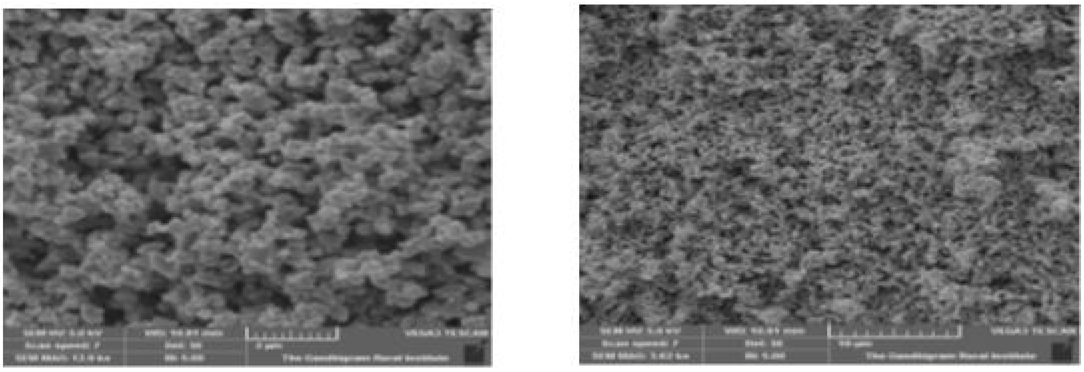

Figure 2: SEM Analysis of Zinc oxide nanoparticles

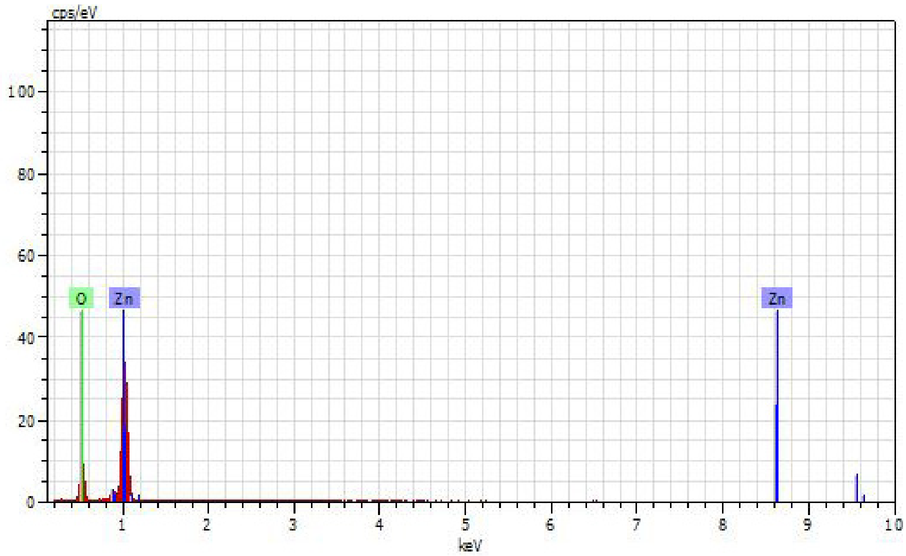

Figure 3: EDAX Image of Zinc oxide nanoparticles

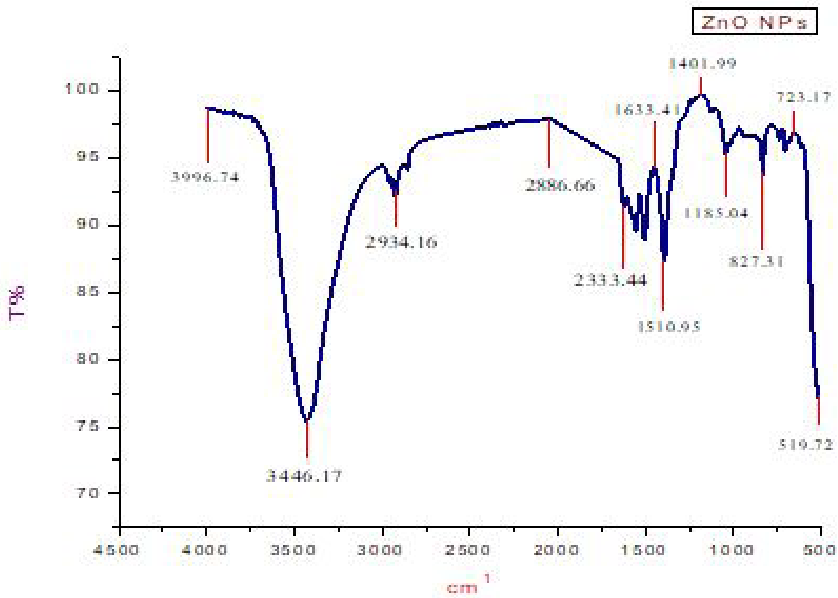

Figure 4: FTIR Image of Zinc oxide nanoparticles

The condition factor of koi carp reared in different feeds were presented as indicated in Table 2. Condition factor (K) of Koi carp was estimated for comparative purposes to assess the feed. The final condition factor of Koi carp was increased in all the feeds supplemented with ZnO nanoparticles. Srinivasan et al., (2016) [13] reported an increase in condition factor of Macrobrachium rosenbergii post-larvae fed with 40g/ kg^{-1} of iron oxide nanoparticles in the feed. A similar condition factor was also reported in common carp fed with iron oxide nanoparticles (Anand Sadanandan Ramya et al.,2015) [14].

Feeds	Initial	Final
Feed I(control)	2.36 ± 0.27	2.41± 0.34
Feed II	2.68 ± 0.15	3.05 ± 0.31
Feed II	2.21 ± 0.09	2.84 ± 0.36
Feed IV	2.34 ± 0.07	2.59 ± 0.35
Feed V	2.81 ± 0.12	3.6 ± 0.35
Feed VI	2.57 ± 0.12	2.96 ± 0.03

Table 2: Condition factor of Koi carp

The different feed utilization and growth parameters are presented in Table 3. The ANOVA (Analysis of Variance) of growth parameters (Feed consumption, Growth, Gross Growth Efficiency, Net Growth Efficiency) are presented in is given (Table 4). The feed consumption was higher in feed IV containing 80mg of zinc oxide nanoparticles. The feed consumption of Mrigal was higher in feed IV containing 15 mg/g^{-1} of zinc oxide nanoparticles (Rajan and Rohini,2021) [9]. The growth was higher in feed VI is (0.79) containing 100mg of zinc oxide nanoparticles. Muralisankar et al., (2016) [15] reported that the growth of Macrobrachium rosenbergii was higher in copper supplemented feed. The analytical variance shows that (ANOVA) the growth is significant (Table 4). Like growth, the percentage growth rate of Koi carp reared in feed VI is higher (18.3). The assimilation of Koi carp reared is higher in feed VI Sangeetha and Rajan (2021) [16] reported that the assimilation of Koi carp was higher in feed IV containing 30 mg of iron oxide nanoparticles in the feed. The metabolism, gross and net growth efficiency of Koi carp was higher in feed VI. Rajan and Rohini (2021) [9] reported that the gross and net growth efficiency was higher in Cirrhinus mrigala fed with feed IV containing 15mg of ZnO NPs in the feed. The analytical variance shows that (ANOVA) the gross and net growth efficiency is significant.

Parameters	EXPERIMENTAL FEEDS					
	Feeds I(control)	Feed II(20 mg)	Feed III(40 mg)	Feed IV(60 mg)	Feed V(80 mg)	Feed VI(100 mg)
Feed consumption(g/g live wt /21 days)	6.7±1.2	5.6±0.75	6.5±0.07	6.7±0.73	7.5±1.1	6.6±1.0
Feed conversion Efficiency	0.28±0.06	0.49±0.10	0.44±0.01	0.50±0.04	0.50±0.11	0.74±0.12
Feed conversion Ratio	14.3±1.5	10.2±1.6	13.2±0.81	11.9±.38	8.8±.71	8.4±1.7
Growth	0.30±0.02	0.45±0.06	0.49±0.03	0.56±0.03	0.62±0.11	0.79±0.06
Percentage Growth	7.1±12.4	12.4±0.85	11.2±.85	12.6±1.5	13.3±4.5	18.3±3.5
Assimilation	1.3±0.28	1.8±0.60	1.9±0.15	3.1±0.20	2.6±0.50	3.2±0.66
Metabolism	1.4±0.62	1.3±0.63	1.4±0.15	2.5±0.20	1.6±0.95	2.4±0.56
Gross Growth Efficiency (%)	4.7±1.1	8.1±1.9	7.5±0.48	8.2±0.42	8.4±0.38	12±0.52
Net Growth Efficiency (%)	21.9±2.3	17.8±0.64	17.9±2.9	21.3±1.2	23.5±1.3	25.2±2.1

Feed Consumption Growth Gross Growth Efficiency Net Growth Efficiency

a vs b (P>0.05)NS a vs b (P>0.05)S a vs b (P>0.05)S a vs b (P>0.05)S

a vs c (p>0.05)NS a vs c (p>0.05)S a vs c (p>0.05)S a vs c (p>0.05)S

a vs d (P>0.05)NS a vs d (P>0.05)S a vs d (P>0.05)S a vs d (P>0.05)S

a vs e (P>0.05)NS a vs e (P>0.05) S a vs e (P>0.05)S a vs e (P>0.05)S

Table 3: Feed utilization and Growth parameters of Koi carp in relation to different quantity of Zinc oxide nanoparticles. Each value is the average (± SD) performance five individuals in triplicates reared for 30 days

Parameters	Source of Varation	Sum of Squares	DF	Mean Squares	F	SIG
Feed Consumption	Between Groups Within Groups Total	5.663 9.773 15.436	5 12 17	1.133 0.814	1.391	0.295 NS
Growth	Between Groups Within Groups Total	0.410 0.045 0.454	5 12 17	0.082 0.004	21.943	0.0005 S
Gross Growth Efficiency	Between Groups Within Groups Total	83.445 31.691 115.136	5 12 17	16.698 2.461	6.319	0.004 S
Net Growth Efficiency	Between Groups Within Groups Total	132.078 44.900 176.978	5 12 17	26.416 3.742	7.060	0.003 S

Table 4: ANOVA (Analysis of Variance) of Growth parameters (Feed consumption, growth, gross growth efficiency, net growth efficiency) of Koi carp

Haematological Parameters

The haematological parameters of Koi carp are presented in Table 5. RBC, haemoglobin, haematocrit and platelets decreased with increased quantity of Zn O NPs and WBC of Koi carp gradually increased from feed I to feed VI. Haematological parameters are very helpful in the judgment of the health condition of fish species. Abdel –Tawwab et al., (2007) [17] reported the increase of blood parameters with a high concentration of selenium nanoparticles supplemented in the feed of African catfish, Clarias gariepinus. Anand

Sadanandan Ramya et al., (2015) [14] reported that the haematological parameters were gradually increased with different doses of iron oxide nanoparticles fed on Indian major carp. Faiz (2015) [18] reported that haematological characteristics of grass carp fed with ZnO supplemented diet showed a significant decrease in WBCs, Hb, HCT values but an increase in RBC. Paria Akbary et al (2018) [19] reported that the Hb, Hct, and RBC counts are decreased and WBC count significantly increased in grey mullet exposed to sub-lethal concentration of copper oxide nanoparticles.

Blood Parameters	Feed I	Feed II	Feed III	Feed IV	Feed V	Feed VI
RBC(millions/cumm)	0.2	0.2	0.13	0.1	0.7	0.03
Hemoglobin (gm/dI)	0.6	0.5	0.4	0.3	0.2	0.1
WBC(cells/cumm)	100	3,200	4,900	5,300	6,100	6,800
Haematocrit (PCV) (%)	1.8	1.7	4.2	3.0	2.6	1.02
Platelets (Lakhs/cumm)	62,000	64,000	58,000	46,000	38,000	29,000

Table 5: Haematological parameters of Koi carp in relation to Zinc oxide nanoparticles incorporated feeds. Each value is average of 5 individual observations

Biochemical Parameters

Total protein, carbohydrate and lipid in muscle, gill and liver of Koi carp is higher in feed V containing 80mg of zinc oxide nanoparticles (Table 6). Thangapandiyan and Monika (2019) [20] reported that the level of carbohydrates present in *Labeo rohita* tissues (muscle, liver, and gills) was gradually increased with an increase in the ZnO NPs concentration. Ashouri et al., (2015) [21] reported that the selenium nanoparticles in the feed increased the protein, carbohydrates and lipid content of muscle, gill and liver on crucian carp. Keerthika et al., (2017) [22] reported that the iron oxide nanoparticles altered the biochemical parameters of *Labeo rohita*.

Feed	Tissues	Prtein(mg/g)	Cabohdrate(mg/g)	Lipid (mg/g)
I	Muscle	1.43	1.20	1.27
	Gill	1.84	0.20	0.93
	Liver	0.60	0.56	0.32
II	Muscle	2.7	1.91	1.02
	Gill	1.63	0.74	0.52
	Liver	0.54	0.53	0.58
III	Muscle	2.89	1.53	1.59
	Gill	1.82	0.98	0.89
	Liver	0.60	0.72	0.62
IV	Muscle	3.14	2.29	1.75
	Gill	1.90	1.29	1.60
	Liver	0.81	0.66	0.91
V	Muscle	3.81	2.52	1.95
	Gill	2.96	1.72	2.0
	Liver	0.93	1.74	1.53
VI	Muscle	3.76	1.98	1.78
	Gill	2.18	1.32	1.69
	Liver	1.37	1.30	1.79

Table 6: Total Protein, carbohydrate and lipid in muscle, gill and liver of Koi carp

Conclusions

The study provides information about the use of ZnO NPs in the feed of koi carp.100mg of ZnO NPs incorporated feed was suitable for the growth of koi carp. Haematological parameters such as red blood corpuscle, haemoglobin, haematocrit and platelets decreased with increased quantity of Zn O NPs and WBC of Koi carp gradually increased from feed I to feed VI. Protein, carbohydrate and lipid in muscle, gill and liver of Koi carp were higher in feed V.

Conflict of Interest

The authors declare no conflict of interest.

Authors Contributions

N. Soundhariya: Done experiments related to the synthesis of zinc oxide nanoparticles, characterization using UV-Vis, SEM, EDAX and FT-IR, collection of fish, feed preparation, feeding trail, estimation of growth, haematological, and biochemical parameters. M.R. Rajan: The research work was formulated and the guidance was given to the first author for execution and written the manuscript.

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References

1. Erkan Can, Volkan Kizak, Murathan Kayim, Safak Seyhaneyildiz Can, BanuKutlu, Mehmet Ates, Mehmet Kocabas, Nida Demirtas(2015) Nanotechnological Applications in Aquaculture-Seafood Industries and Adverse Effects of Nanoparticles on Environment. J Mater Sci Engin 5: 605-609.

2. Onuegbu CU, Aggarwal A, Singh NB (2018) ZnO nanoparticles as feed supplements on growth performance of cultured African catfish fingerings. J Sci Indus Res 77: 213 – 218.

3. Lall SP(2002)The minerals. Fish Nutr 3:259-308.

4. Lowry OH, Rosenbrough NJ, Farr AL(1951)Protein measurement with the Folin Phenol Reagent. J Biol Chem 193(1): 265–275.

5. Carrol NV, Longley RW, Roe JH(1956)Glycogen determination in liver and muscle by use of anthrone reagent. J Biol Chem 220(2): 583–593

6. Barnes H, Blackstock J. (1973) Estimation of lipids in marine animals and tissues detailed investigation of the sulphophosphovanillin method for total lipids., J.Exp.Mor.Bio.Ecol. 12: 103-118.

7. Jamdagni P, Singh Rana J, Khatri P, Nehra K(2018) Comparative account of antifungal Activity of green and chemically synthesized Zinc Oxide nanoparticles in combination with agricultural fungicides. Inter J Nano Dim 9: 198-208.

8. Ahmadi Shadmehri A, Namvar F, Miri H, Yaghmaei P, Nakhaei Moghaddam M. (2019) Assessment of antioxidant and antibacterial activities of Zinc Oxide nanoparticles, Graphene and Graphene decorated by Zinc Oxide nanoparticles. Inter J Nano Dim 10 (4): 350-358.

9. Rajan MR, Rohini R(2021) Impact of different quantity of Zinc oxide nanoparticles on growth and hematology of Mrigal Chirrhinus mrigala. J Water Environ Nanotechnol 6(1):62-71.

10. Jeyabharathi S, Kalishwarlal K, Sundar K, Muthukumaran A(2017) A Synthesis of zinc Oxide nanoparticles by aqueous extract of Amaranthus caudatus and evaluation of their toxicity and antimicrobial activity. Matt Lett 209:295-298.

11. Sadhan Kumar Chaudhuri, Lalit Malodia(2017) Biosynthesis of zinc oxide nanoparticles using leaf extract of Calotropis gigantean: characterization and its evaluation on tree seedling growth in nursery stage. Appl Nanosci7:501- 512.

12. Purwaningsih SY, Pratapa S, Triwikantoro, Darminto (2015) Synthesis of Nanosized ZnO particles by co-precipitation method with variation of heating time.6th Nanoscience and Nanotechnology symposium, AIP Conf. Proc.1710:03040-1-030040-6.

13. SrinivasanV, Saravana Bhavan P, Rajkumar G, Satgurunathan T, Muralisankar T (2016) Effects of dietary iron oxide nanoparticles on the growth performance, biochemical constituents and physiological stress responses of the giant freshwater prawn Macrobrachium rosenbergii post larvae. Inter J Fish Aqu Stud 4(2):170182.

14. Anand Sadanandan Ramya, Mathan Ramesh, Manoharan Saravanan, Rama Krishnan Poopal, Subramanian Bharathi, Devaraj Nataraj (2015) Iron oxide nanoparticles to an Indian major carp, Labeo rohita: Impacts on hematology, iono regulation and gill Na+/K+ ATPase activity. J King Saud Univ Sci 27: 151-160.

15. Muralisankar T, Saravana Bhavan P, Radhakrishnan S, Seenivasan C, Manickam N, Srinivasan V (2016) Effects of dietary zinc

on the growth, digestive enzymes activities, Muscle biochemical compositions and antioxidant status of the giant freshwater prawn Macrobrachium rosenbergii. Aquacul 448 : 98-104.

16. Sangeetha K, Rajan MR(2021) Evaluation of Different Quantity of Iron Oxide Nanoparticles on Growth, Haematological and Biochemical Characteristics of Koi Carp. Agricultural Science Digest - A Res J Agri Ani Vet Sci 41(2): 338-344.

17. Abdel-Tawwab M, Mousa MA, Abbas FE (2007) Growth performance and Physiological response of African cat fish, Clarius gariepinus (B) fed organic selenium prior to exposure to environmental copper toxicity. Aquacult 272(1): 335-345.

18. Hina Faiz, Amina Zubari, Samina Zazir, Mohammed Rauf, Naima Younas(2015) Zinc oxide, zinc sulphate, and zinc oxide nanoparticles as source of dietary zinc; comparative effects on growth and haematological indices of juvenile grass carp. Int J Agric Biol 17(3): 568-574.

19. Paria Akbary, Sahar Sartipi Yarahmadi, Abdolreza Jahanbakhshi(2018) Haematological, hepatic enzymes activity and oxidative stress responses of gray mullet (Mugil cephalous) after sub-acute exposure to copper oxide. Environ Sci Poll Res 25:1800-1808.

20. Thangapandiyan S, Monika S(2020)Green Synthesized Zinc Oxide Nanoparticles as Feed Additives to Improve Growth, Biochemical, and Hematological Parameters in Freshwater Fish Labeo rohita. Biol Tra Ele Res 195(2):636-647.

21. Ashouri S, Keyvanshokooh S, Salati AP, Johari SA, Zansoosi HA (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). J Aqua 446(1): 25-29.

22. Keerthika V, Ramesh R, Rajan MR (2017) Toxicity assessment of iron oxide nanoparticles in Labeo rohita. Inter J Fish Aqua Stu 5(4): 01-06.