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Different quantities of copper oxide nanoparticles incorporated feed on growth and haematological parameters Tilapia *Oreochromis mossambicus*

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Abstract

Copper oxide nanoparticles were synthesized and characterized using by UV-Visible Spectroscopy, Scanning Electron Microscope, EDAX, X-Ray Diffraction and Fourier Transform Inferior Spectroscopy. Six feeds were prepared by using a fish meal, groundnut oil cake, wheat flour tapioca flour and different quantities of copper oxide nanoparticles such as 0,20, 40, 60, 80 and 100 mg/100g of feed. Feed utilization and haematological parameters were estimated after 21 days. The UV-visible absorption spectra show that CuO NPs were measured in wavelengths within 200 to 300 nm and exhibited strong adsorption at 220 nm. SEM images show that the Copper oxide nanoparticles were observed at the wavelength range of 9.18 nm. EDAX spectrum recorded two peaks located on the spectrum at 0.9 KeV and 8 KeV. XRD images were observed at the wavelength range from 75 nm. FT-IR spectrum was observed at the wavelength range from 400-4000 cm. The final condition factor of Tilapia was higher when compared to the initial. The feed consumption of Tilapia is higher in feed V (8.03) and lower in feed I (4.83). The feed conversion efficiency of Tilapia was higher in feed VI (7.6±6.9) and lower in feed IV (2.4+1.4). The feed conversion ratio of Tilapia was best in feed IV. Growth and the percentage growth of Tilapia were higher in feed III and I respectively. Assimilation of Tilapia was higher in feed IV and lower in feed V. Metabolism of Tilapia was higher in feed VI and lower in feed II. The WBC count is gradually increased from feed I to feed VI. Haemoglobin content decreased from feed I to feed VI. Haematocrit (Het) is higher in feed I (1.5%) and lower in feed VI (0.3 %). The platelets are increased with an increasing quantity of copper oxide nanoparticles in the feed.

Keywords: Different; Copper oxide; Nanoparticles; Feed; Growth; Haematological

1. Introduction

Nanoparticles possess unique properties such as large surface area and consequently greater reactions than macro-sized particles. Therefore, questions concerning its potential adverse effect on human and environmental health often have been raised and are being widely used in consumer products and are thus expected to find their way into aquatic, atmospheric and terrestrial environments. Nanoparticles such as Se, Cu, Fe, FeO, Zn and CuO play a vital role in aquaculture operations and these are essential microminerals to enhance fish growth. This dietary supplementation of nanoparticles produces better survival, growth, antioxidant levels, and immunity in aquatic organisms. Copper is an essential component of numerous oxidation-reduction enzyme systems, such as cytochrome c oxidase, uricase, tyrosinase, superoxide, dismutase, amino oxidase, and caeruloplasmin. It is intimately involved with iron metabolism and therefore hemoglobin synthesis, red blood cell production, and maintenance. Copper is used in aquaculture for various purposes, including control of the blue-green algae responsible for off-flavors in culture animals, treating certain diseases and parasites, eliminating mollusks from ponds, and avoiding fouling of fish cage netting. Copper is also believed to be necessary for the formation of the pigment melanin and consequently skin pigmentation for the formation of bone and connective tissue for maintaining the integrity of the myelin sheath of nerve fibers. Copper is also needed

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for normal connective tissue metabolism and functions with the central nervous system [1]. The haematological analysis is excellent to assess the stress condition of aquatic organisms [2] Tilapia is the economically most important species inhabiting shallow, streams, ponds, rivers, and lakes less commonly found in brackish water. The work related to different quantities of CuO nanoparticle incorporated feed on growth and haematological parameters of tilapia is wanting. Hence the present study was carried out.

2. Material and methods

2.1. Experimental fish

Tilapia are mainly freshwater fish inhabiting shallow streams, ponds, rivers, and lakes and are less commonly found living in brackish water. Tilapia is an inexpensive, mild-flavored fish. It is the fourth most commonly consumed type of fish in India.

2.2. Synthesis of copper oxide nanoparticles

The CuO nanoparticles were synthesized by the chemical reduction process using copper sulfate pentahydrate as precursor salt with a starch capping agent. The preparation method starts with the addition of 0.1M (92.49g) copper sulfate pentahydrate solution taken in a beaker and 120 ml of starch (1.2%) (8.81g) solution was added with vigorous stirring and heating at 60 ^{0}c for 30 minutes. Then 50ml of 0.2M (1.76g) ascorbic acid solution under continuous rapid stirring. Subsequently, 30 ml of 1M (1.2 g) sodium hydroxide solution is slowly added to the prepared solution with constant stirring and heating 80^{0}c for 2 hours. The color of the solution turned yellow to ocher. After completion of the reaction, the solution was taken from the heat and allowed to settle overnight and the supernatant solution was discarded. The precipitate was separated from the solution by filtration and washed with deionized water and ethanol three times to take out the excessive starch bound with nanoparticles. Ocher color precipitate was obtained and dried at room temperature. After drying, nanoparticles were obtained and stored in a glass vial for further studies.

2.3. Collection of fish

For the present work, Tilapia juveniles (1.5±0.05g) were collected from K.V.K fish farm Palani, Tamil Nādu, India, and transported to the laboratory in polythene bags filled with oxygenated water and acclimated in the plastic through for 15 days at 28±2^{0}C. During acclimation, fish were fed with trainee feed containing fish meal, GNOC, wheat flour, and rice bran in form of dry pellets.

2.4. Selection of Feed Ingredients and Experimental Feed Preparation

The raw materials for feed preparation were selected based on their ability to supply nutrients and are indicated in table 1. After knowing the protein content by the Micro-Kjeldhal method, the feed was prepared. Fish meal and groundnut oil cake were used as protein sources; wheat flour and tapioca flour were used as carbohydrate sources; vegetable oil was used as lipid sources; Supplevite mix was also added. The components used for feed preparation were dried, powdered, and sieved through a 425-micron sieve. The ingredients were weighed and mixed thoroughly with 130-150ml of distilled water. The mixed feedstuff was put in the autoclave for 15 minutes at 100^{0}C and cooled. After cooling, fish oil, sunflower oil, supplevite mix, sodium chloride, sodium benzoate, and different quantity of copper oxide nanoparticles (20,40,60,80,100mg/g-1) were mixed with feed and it was extruded with the help of a pelletizer. The pellet was dried at room temperature. The formulated feed was kept in an airtight container at 20^{0}C until use to prevent contamination (Table 2).

Table 1 Ingredients used in the feed and protein content

Sr. No	Ingredients	Protein Content (%)
1	Fish meal	58
2	Groundnut oil cake	44
3	Wheat flour	11
4	Tapioca	03
5	Fish oil	-
6	Sunflower oil	-
7	Supplevite-mix*	-
8	Sodium chloride	-
9	Sodium benzoate	-

Table 2 Composition of Different Ingredients in the Experimental Feed (g/100gm) of Tilapia

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	11.2	11.2	11.2	11.2	11.2	11.2
Tapioca	11.2	11.2	11.2	11.2	11.2	11.2
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
Sodium Benzoate	2	2	2	2	2	2
CuO nanoparticles	0	20mg	40mg	60mg	80mg	100mg

2.5. Characterization of Copper Oxide Nanoparticles

2.5.1. UV-visible spectroscopy (UV-Vis)

UV-visible spectroscopy measures the extinction and absorption of light passing through a sample. Nanoparticles have unique optical properties that are sensitive to the size, shape, concentration, agglomeration state, and refractive index near the nanomaterials. The most important factor of the technique is to give information about the material when light falls on it.

2.5.2. Scanning Electron Microscope (SEM)

Sem analysis is a powerful investigative tool that uses a focused beam of electrons to produce complex high-magnification images of a sample's surface topography. The morphology of the copper oxide was investigated using a scanning electron microscope (SEM) (LEO1455VP)

2.5.3. Energy dispersive X-ray Spectroscopy (EDAX)

A minute drop of nanoparticles solutions was last aluminum foil and subsequently dried in the air before transferring to the microscope on energy dispersive X-ray detection instrument (EDAX) (HORIBA8121-4) was used to examine the elemental composition of the CuO nanoparticles.

2.5.4. X-ray diffraction (XRD)

X-ray powerful diffraction (XRD) is a rapid technique primarily used for phase identification of a given material and can provide information on unit cell dimensions. The analyzed material is finely ground and homogenized and the average bulk composition is determined.

2.5.5. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR is a very versatile tool for the surface characterization of nanoparticles and provides a specific condition, the chemical composition of the nanoparticles, surface determined and the surface reactive sites responsible for the surface reactivity can be identified. In addition, chemical reactions taking place at the nanoparticle's surface can be monitored in situ as a function of various parameters, such as temperature and gaseous environment.

2.6. Experimental design for growth studies

For the present study, uniform sizes of tilapia (1±0.5g) were selected and then the fishes were introduced in the through having a capacity of 15 liters. The initial length and weight of the fish were taken. Ten fishes were introduced in each trough. For each treatment triplicates were maintained. During rearing, the fish were fed on an ad-libitum diet of the prepared feed twice a day for 1 hour each from 9-10 am and 4.5pm. 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 with the least disturbance to the fish and dried at 95^{0}C. Approximately 70% of the water in the tank was replaced with tap water. The experiment was continued for 21^{st} days. On the 21^{st} day length and weight of the fish were taken. Condition factor(K) and Feed utilization parameters such as feed consumption, Feed conversion Efficiency, Feed Conversion Ratio, Growth, Percentage Growth, Relative Growth Rate, Assimilation, Metabolism, Gross Growth Efficiency, and Net Growth Efficiency were estimated.

2.7. Hematological parameters

Blood samples were collected from fish after 21 days, from the cardinal vein on the right side of the fish with a disposable insulin syringe fitted with the fine needle, without harming the fish. The syringe and needle were moistened with EDTA. The collected blood was then transferred into an Eppendorf tube containing 0.1 N EDTA. Complete blood parameters such as RBC, WBC, Platelet count, Haemoglobin, Hematocrit, Mean corpuscular volume, Mean corpuscular haemoglobin, and Mean corpuscular haemoglobin concentration were estimated after 21 days

3. Results and discussion

The chemical synthesized CuO NPs were characterized by UV – Visible absorption spectrum and the copper oxide nanoparticles were measured in a wavelength range from 200-300nm. (Figure1) The sharp bands indicate the formation of CuO NPs [3]. The morphology of the material was studied through scanning electron microscopy. SEM indicates the spherical shape of synthesized copper oxide nanoparticles (Figure 2). Also reported that the mean particle size of CuO is about 50-70nm and the particle have good homogeneity, spherical structure, and appropriate separation [4]. The presence of oxygen (o) and copper (Cu) was revealed in the synthesized nanoparticles and shown as two peaks located between 0.4 KeV and 0.9 KeV. The two peaks indicated the purity of copper oxide nanoparticles and were located on the spectrum at 0.9 KeV and 8 KeV and another peak of 0 elements was located the at 0.5 KeV. EDAX spectrum copper oxide nanoparticles were recorded which spectrum with no other materials peaks indicating the purity of the copper oxide nanoparticles (Figure 3). The Energy-dispersive X-ray Analysis of the CuO nanoparticles and the data indicate that the nanopowders are nearly stoichiometric [5]. The XRD diffraction peaks are indexed as 36.4308, 43.3079, 50.4343,74.1074 which is represented in (Figure 4). All diffraction peaks are indexed according to the hexagonal phase of copper oxide nanoparticles (JCPDFNO.36-1451) no characteristic peak impurity phase except the crystalline structure of CuO nanoparticles was found to be 75nm. Gangarapu Manjari et al., (2017)[6] reported that the CuO nanoparticles monoclinic phase and crystalline nature (JCPDS-05-0661). The FT-IR spectrum of copper oxide nanoparticles was analyzed in the range of 400-4000cm-1. The FT-IR measurement was carried out for identifying the functional groups of bioactive components based on the peak value in the region of infrared radiation. Copper oxide formation was confirmed 462.33nm bands have 3422.12, 2919.73, 2304.55,1628.59, 1381.14, 1220.71, 1026.91, were are associated with c-o alcohol, N=O Nitro group, C=CO Carbonyl, C-CL Alkaline Halide, C-N Amine (Figure 5). The FTIR measurements for the synthesized copper oxide NPs help in identifying possible biomolecules that act as reducing and capping agents [7]. The strong peaks observed at 3441, 1633, 1046 and 1403 cm-1 correspond to OH, C-C, C-O and aliphatic C-H stretching vibrations respectively. The peaks at 620 and 686 cm-1 correspond to Cu (1)-O vibration of copper oxide nanoparticles.

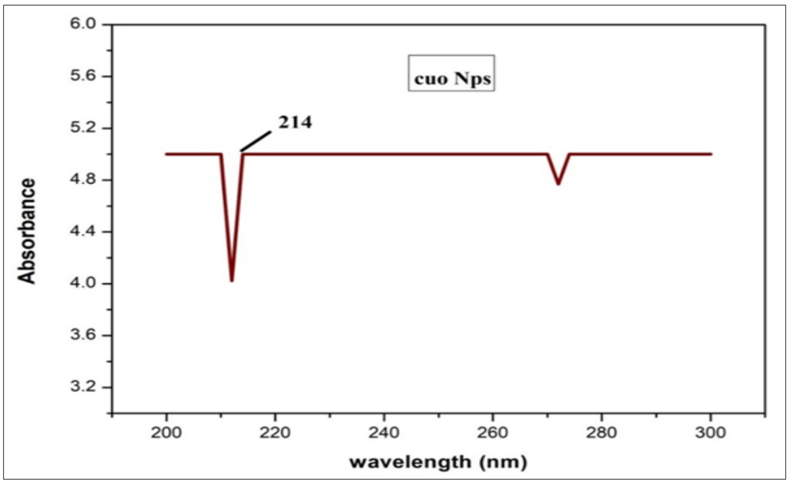

Figure 1 UV-Visible Spectroscopic Analysis of Copper Oxide nanoparticles

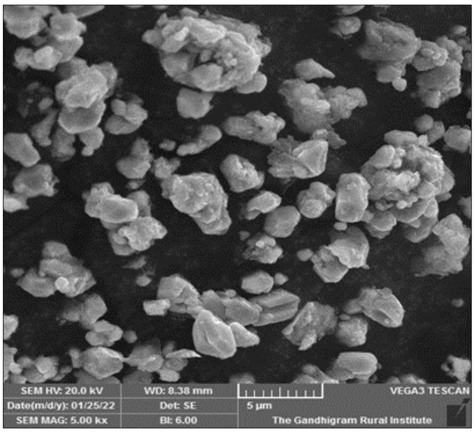

Figure 2 SEM Analysis of Copper Oxide nanoparticles (5µm)

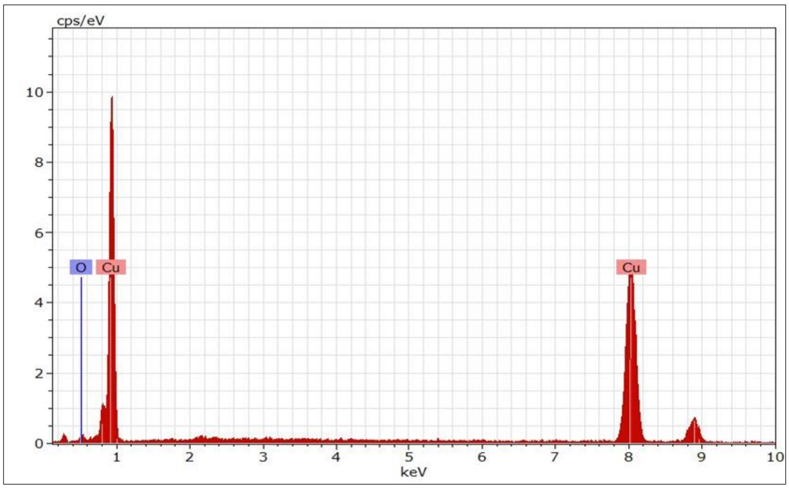

Figure 3 EDAX Image of Copper Oxide nanoparticles

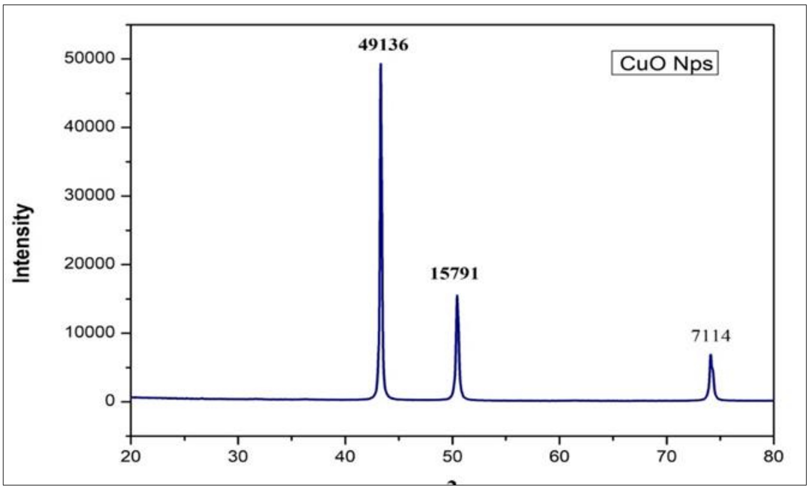

Figure 4 XRD Analysis of Copper Oxide Nanoparticles

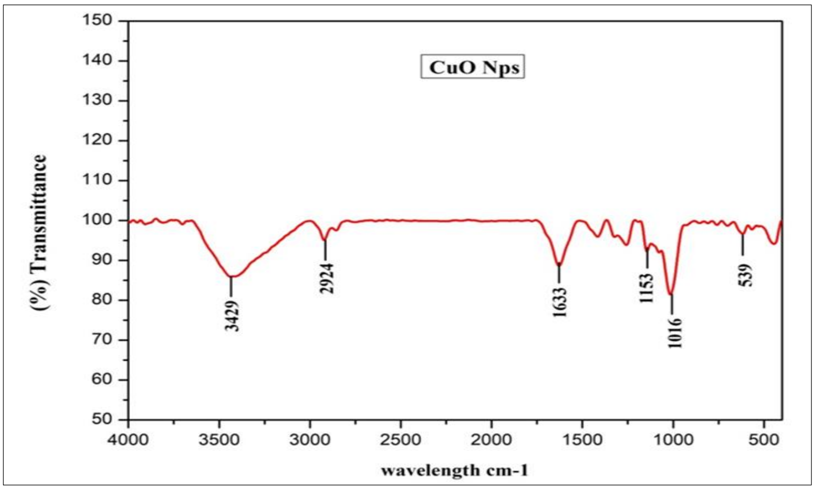

Figure 5 FT-IR Image of Copper Oxide nanoparticles

The condition factor of tilapia reared in different feeds were presented in figure 6. The final condition factor is increased in all feeds. An increase in the condition factor of *Macrobrachium rosenbergii* post-larvae fed with 40g/ kg-1 of iron oxide nanoparticles in the feed was reported[8].

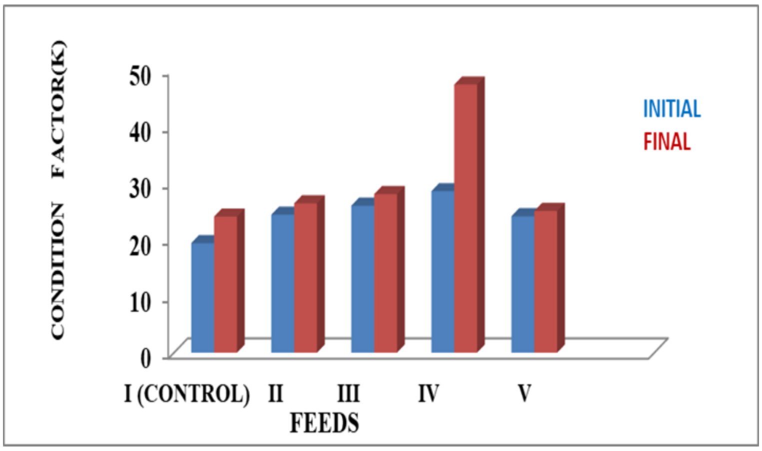

Figure 6 Condition factor (k) of Tilapia

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) are presented in table 4. The feed consumption of tilapia reared in feed I (control) was 4.83g/g live wt/21 days. The feed consumption was higher in feed V (8.03) containing 80 mg of copper oxide nanoparticles. An increase in the concentration of zinc oxide nanoparticles with feed consumption in African Catfish fingerlings [9]. The feed conversion efficiency of tilapia reared in feed I is 6.8. Also reported that Mrigal's feed consumption and conversion efficiency was higher in feed VI containing 100 mg of ZnO NPs in the feed [10]. The feed conversion ratio was best in feed IV (2.4). The feed conversion ratio was best in control and lower in zinc oxide fed of *Macrobrachium rosenbergii* [1]. The growth was higher in feed III containing 40 mg of copper oxide nanoparticles. The analytical variance (ANOVA) shows that the growth is significant. The growth rate gradually increased in lower concentrations to higher concentrations of zinc-supplemented feed of *Penaeus vannamai* [11]. The percentage growth rate of tilapia reared in feed II is higher than 7.3. Like growth, the relative growth rate in feed III was higher. The assimilation and metabolism of tilapia reared in feed V were higher. The assimilation of Koi carp was higher in feed IV containing 30 mg of iron oxide nanoparticles in the feed [12]. The gross and net growth efficiency of tilapia reared in feed III was higher. The analytical variance (ANOVA) shows significant gross and net growth efficiency. Koi carp's gross and net growth efficiency was higher in feed VI containing 100 mg of ZnO NPs in feed [13].

Table 3 Feed utilization and Growth parameters of Tilapia in relation to different quantities of copper oxide nanoparticles. Each value is the average (±SD) of 10 fishes in triplicate

Parameters	Experimental feeds					
	Feed 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)	4.83±0.9	5.6±0.4	6.7±0.4	5.8±1.0	8.03±1.9	6.7±1.9
Feed conversion Efficiency	6.8±1.82	7.5±1.82	1.03±0.88	2.4±1.4	2.6±1.6	7.6±1.9
Feed conversion Ratio	3.06±1.73	1.78±0.21	1.1±0.95	2.53±1.39	5.31±3.75	3.75±2.86
Growth	0.5±0.4	1±0.3	2.6±0.8	1.4±0.4	1.5±0.3	2.3±0.3
Percentage Growth	21±0.17	73±0.50	34±.0.08	8±0.02	12±0.07	15±0.05
Relative Growth Rate	0.26±0.20	0.33±.0.15	0.43±0.07	0.23±0.05	0.16±0.05	0.18±0.05
Assimilation (g/g live wt/21 days)	0.35±0.3	0.28±0.12	0.39±0.11	0.39±0.1	1.19±0.4	0.64±0.1
Metabolism (g/g live wt/21 days)	0.35±0.26	0.38±0.35	0.48±0.02	0.07±0.08	0.86±0.3	0.27±0.1
Gross Growth Efficiency (%)	46.8±35.5	82.3±68.6	96.6±30.1	56.3±18.2	18.6±2.08.	34.8±20.06
Net Growth Efficiency (%)	21.9±13.5	17.9±11.6	25.5±20.3	23.2±19.3	6.66±7.9	23.1±20.04

Table 4 ANOVA (Analysis of Variance) of Growth parameters (Feed Consumption, Growth, Gross Growth Efficiency, Net Growth Efficiency) of Tilapia *Oreochromis mossambicus*

Parameters	Source of Variation	Sum of Squares	df	Mean Squares	F	Sig
Feed Consumption	Between Group	6.694	5	1.339	1.668	.217
	Within Group	9.631	12	0.803		NS
	Total	16.324	17			
Growth	Between Group	.946	5	.189	10.286	0.01
	Within Group	.221	12	.018		S
	Total	1.167	17			
Gross Growth Efficiency	Between Group	581.344	5	116.269	33.715	.000 S
	Within Group	41.383	12	3.449		
	Total	622.727	17			
Net Growth Efficiency	Between Group	878.944	5	175.789	47.942	.000
	Within Group	44.000	12	3.667		S
	Total	922.944	17			

The hematological parameters of tilapia are presented in table 5. The WBC, RBC and haemoglobin of tilapia are gradually decreased from feed I to VI. A similar decrease was reported in Zebrafish fed with ZnO nanoparticles incorporated in feed [14]. Hematocrit is higher in the feed I (1.5%) and lower in feed V and VI (0.3%). The platelets increased with the increasing quantity of copper oxide nanoparticles. Such higher hematocrit and platelets were also reported in iron oxide nanoparticles incorporated feed in Mrigal[15]. The haematological characteristics of grass carp fed with ZnO supplemented diet showed a significant decrease in WBCs, Hb, HCT, MCV, and MCH values but an increase in RBC and MCHC values [16].

Table 5 Hematological parameters of Tilapia Exposed to Copper Oxide Nanoparticles

S.No	Parameters	Control	Feed I	Feed II	Feed III	Feed IV	Feed V
1	WBC (Cells/cumm)	3500	2400	1900	1600	1400	1200
2	Haemoglobin (gm/dL)	0.5	0.4	0.3	0.2	0.1	0.1
3	RBC Count (Millions/cumm)	0.20	0.13	0.10	00.1	0.03	0.03
4	Haematocrit (%)	1.5	1.2	1.0	0.6	0.3	0.3
5	Platelets Count (Lakhs/cumm)	24000	17000	23000	30000	36000	42000

4. Conclusion

The present study concludes that feed III containing 40 mg/100 g of feed is optimum for the growth of tilapia and all the haematological parameters decreased with the increasing quantity of copper oxide nanoparticles in the feed.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest among the authors.

Statement of ethical approval

Fish used as an animal model in the present study were 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 Welfare 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.

Author's contributions

M.R.R. designed and supervised the second author for execution. P.P. synthesized and characterized the copper oxide nanoparticles, collected the fishes, performed the growth studies, and estimated the feed utilization and haematological parameters of Tilapia. All the authors read and approved the final manuscript.

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