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Article

# Evaluation of disparate multiplicities of copper oxide nanoparticles integrated feed on the growth and hematology of koi carp

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**Abstract:** The aim of the present study was to assess the effects of different multiplicities of integrated feed containing copper oxide nanoparticles (CuO NPs) on the morphology and growth of Koi carp. UV-visible spectroscopy (UV-Vis), scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDAX), X-ray diffraction (XRD), and Fourier transform infrared spectroscopy (FT-IR) were used to produced and characterize the CuO nanoparticles. Different copper oxide nanoparticle multiplicities, including 25, 50, 100, 150, and 200 mg, were added to the 100 g feed. After 21 days, the Koi carp's feed consumption and hematological parameters were assessed. CuO NPs were examined at 200–300 nm wavelengths, according to the UV–visible absorption spectra. At wavelengths ranging from 9.18 mm (scale bar 10 μm) to 9.18 mm (scale bar 2 μm), SEM pictures were observed. Two peaks in the spectrum at 0.9 KeV and 8 KeV were detected by the EDAX spectrum. The wavelength range for the XRD picture observation was 75 nm. The 400 to 4000 $cm^{-1}$ wavelength range was used to observe the FT-IR spectra. The Koi carp's condition factor and feed utilization metrics were greater in feed VI. From feed I to feed VI, all other parameters are gradually reduced while the WBC count is steadily increased. The study came to the conclusion that Koi carp might grow well with 200 mg of copper oxide nanoparticles mixed with diet.

**Keywords:** copper oxide; different feed; growth; hematology; quantities

## 1. Introduction

Nanoparticles acquired unique properties such as greater surface area and consequently more reactivity than macro-sized particles. Therefore, questions concerning its possible adverse effects on human and environmental health very often have been increased and are being widely used in consumer products and are thus expected to find their way into aquatic, terrestrial, and atmospheric environments, where their fate and behaviour are unpredictable [1]. An essential aspect of aquaculture systems are nanoparticles including selenium, copper, iron, iron oxide, zinc, and copper oxide, which are microminerals that greatly promote fish growth. The dietary supplementation of nanoparticles causes better survival, growth, antioxidant levels and enhancement of immunity in aquatic species. Copper is a necessary component of a multitude of oxidation-reduction enzyme systems, such as cytochrome c oxidase, uricase, tyrosinase, superoxide dismutase, and amine oxidase. It is a strong connection with iron metabolism and, therefore, haemoglobin synthesis, as well as in red blood cell formation and maintenance. Additionally, believed that copper is required for the production of bone and connective tissue, the construction of the integrity of the myelin sheath around nerve fibres, and the production of the pigment melanin, which results in skin pigmentation. Furthermore,

copper is required for healthy connective tissue metabolism and central nervous system operations [2,3]. There is a lack of research on the effects of varying multiplicities of copper oxide nanoparticles integrated feed on the growth and haematological traits of Koi carp (*Cyprinuscarpio var. koi*). This is because the current work was done.

## 2.Materials and methods

### 2.1. Synthesis of copper oxide nanoparticles

The chemical reduction method was used to synthesis the copper nanoparticles, utilizing a starch capping agent and copper sulphate pentahydrate as the precursor salt. The synthesizing process begins with adding 120 mL of a 1.2% (8.81 g) starch solution to a beaker containing 0.1 M (2.49 g) copper sulphate pentahydrate solution. The mixture is then vigorously stirred and heated to 60 ℃ for 30 min. Thereafter, while continuously stirring, 50 mL of a 0.2 M (1.76 g) ascorbic acid solution was added to the synthesis solution step by step. Afterwards, 30 mL of a 1 M (1.2 g) sodium hydroxide solution was gradually added to the mixture, stirred continuously, and heated for two hours at 80 ℃. The solution's colour changed from yellow to ocher. Following the reaction's conclusion, the mixture was removed from the hot plate, allowed overnight to settle, and the supernatant solution was disposed. To remove the excess starch bound with nanoparticles, the precipitate was filtered out of the solution and then 3 times washed thoroughly with deionized waterand ethanol. Precipitates with an ochre colour were obtained, and they were allowed to air dry. Following drying, the obtained nanoparticles were stored in a glass vial for various studies.

### 2.2. Copper oxide nanoparticles: Characterization

The following techniques were used to characterize the synthesised copper oxide nanoparticles.

#### 2.2.1. UV-Visible spectroscopy

UV-VIS spectroscopy was used to investigate as the primary characterization of synthesized copper oxide nanoparticles. The Spectra UV-VIS Double Beam DUV 3500 automated spectrophotometer was used for this analysis.

#### 2.2.2. Scanning electron microscope

SEM analysis is a potent investigative technique that generates accurate, high-magnification images of a sample's surface topography using a focused electron beam. The copper oxide nanoparticles morphology was examined through the use of a scanning electron microscope (SEM) (LEO 1455 VP).

#### 2.2.3. Energy dispersive X-ray spectroscopy

A small droplet of the nanoparticle solution was placed onto aluminium foil, allowed to dry in the air, and then placed under the microscope. The elemental composition of the copper oxide nanoparticles was examined using an energy-dispersive X-ray detection equipment (HORIBA 8121-H).

### 2.2.4. X-ray diffraction analysis

To analyse the nanoparticle's crystalline structure, X-ray diffraction was performed using a powder diffractometer (Rigaku III/A, Japan) equipped with a nickel filter and a copper oxide target.

### 2.2.5. Fourier transform infrared spectroscopy

Fourier Transform Infrared Spectroscopy study utilizing JASCO (FTIR-6200) Spectra was used to examine the vibration modes of the functional group of copper oxide nanoparticles. Different functional groups are absorbed in response to the vibrational energy of the chemical bond based on the characteristic frequency.

## 2.3. Collection and acclimation of Koi carp

In this study, fingerlings of Koi carp (*Cyprinuscarpio var. koi*) weighing 1 ± 0.05 g were procured from Arjun Fish Farm in Madurai, Tamil Nadu, India, and transported to the lab using oxygenated water-filled polythene bags. For 15 days at 28 ± 2 ℃, the fish were acclimated in the plastic trough. Fish meal, groundnut oil cake, wheat flour, and rice bran in the form of dry pellets were given to the fish as they acclimated.

## 2.4. Selection of feed components and preparing experimental feed

The selection of raw materials for feed preparation was done on the basis of their potential supply nutrition. The feed was prepared after the key ingredients' protein contents were determined using the Micro-Kjeldhal technique (**Table 1**). The protein sources were groundnut oil cake and fish meal; the carbohydrates were tapioca and wheat flour; the binding agent was vegetable oil; and the supplevite mix was added. The feed preparation ingredients were dehydrated, ground into a powder, and passed through a 425-micron filter (**Table 2**). After weighing each item, 130–150 mL of distilled water was added and carefully mixed. After being autoclaved for 15 minutes at 100 ℃, the mixed feed material was cooled. A pelletizer was used to extrude the feed after it had cooled and combined with fish oil, sunflower oil, supplevite mix, sodium chloride, sodium benzoate, and copper oxide nanoparticles (25, 50, 100, 150, and 200 $mg/g^{-1}$). At room temperature, the pellet was dried. Until it was used, the prepared feed was stored at −20 ℃ in an airtight container to avoid contamination.

**Table 1.** Protein content of major ingredients.

| S.No | Ingredients | Percentage of protein |
|---|---|---|
| 1 | Fishmeal | 58 |
| 2 | Groundnut oilcake | 44 |
| 3 | Wheat flour | 11 |
| 4 | Tapioca | 03 |

**Table 2.** Contents of various ingredients in Koi carp experimental feed (g/100 gm).

| | **Experimental feeds** | | | | | |
|---|---|---|---|---|---|---|
| **Ingredients** | **I (Control)** | **II (25 mg)** | **III (50 mg)** | **IV (100 mg)** | **V (150 mg)** | **VI (200 mg)** |
| Fishmeal | 33.75 | 33.75 | 33.75 | 33.75 | 33.75 | 33.75 |
| GNOC (Groundnut Oil Cake) | 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 |
| Copper oxide nanoparticles | 0 | 25 mg | 50 mg | 100 mg | 150 mg | 200 mg |

## 2.5. Designing an experiment for growth studies

A uniform size of *Cyprinuscarpio* (1 ± 0.05g) was chosen for the current study, and the fish were thereafter placed in a 15-liter trough. The fish's initial length was measured. Each trough was stocked with ten fish. Each treatment was recorded in triplicate. The fish were fed an ad-libitum diet of the prepared feed twice a day for an hour each, between 9 and 10 am and 5 pm, during the rearing process. After feeding the fish for an hour, the unfed were gathered without disturbing them. Dried the unfed to a constant weight. Every day, before changing the water, the faeces were collected in the least disruptive way possible and dried at 95 ℃. Tap water replaced about 70% of the experimental water in the tank. For 21 days, the experiment was conducted. The fish were measured in live conditions for weight and length on the 21st day.

## 2.6. Growth and feed utilization parameters

After 21 days, feed utilization and growth characteristics were calculated, including condition factor, feed consumption, feed conversion efficiency, feed conversion ratio, growth, percentage growth, relative growth rate, assimilation, metabolism, and gross and net growth efficiency.

## 2.7. Parameters related to haematology

After 21 days, blood was taken from the fish without causing any harm to them, using a disposable insulin syringe fitted with a thin needle from the caudal vein of the fish. EDTA was used to wet the needle and syringe (an Anticoagulant). After blood collection, the sample was placed in an Eppendorf tube with 0.1 N EDTA. Following 21 days, estimates were made for all complete blood parameters, including RBC, WBC, Platelet count, haemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC).

## 3. Results

The chemical synthesized CuO NPs were characterized by UV-visible analysis presented in **Figure 1**. The UV-visible absorption spectrum of copper oxide nanoparticles was measured in wavelength 200–300 nm. The sharp bands were observed to 220 nm throughout the reaction which indicates the formation of CuO NPs. The morphology of the material was studied through scanning electron microscopy. That indicates the spherical shape of synthesized copper oxide nanoparticles (**Figure 2**).

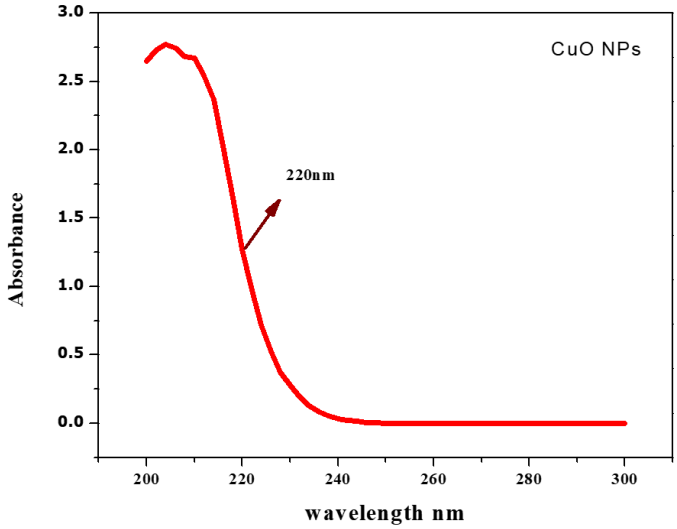


**Figure 1.** UV-Visible Spectroscopy of CuO NPs.

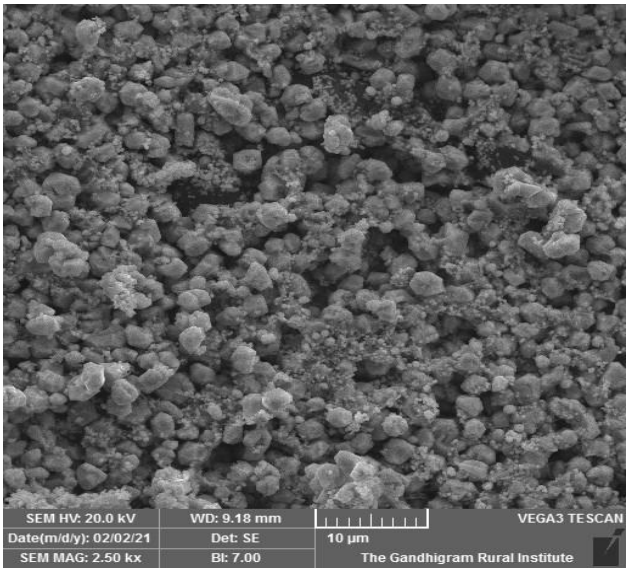


**Figure 2.** SEM image of CuO NPs.

UV-visible analysis, as characterized in, was used to characterize the chemically synthesized CuO NPs (**Figure 1**). The wavelength range of 200–300 nm was used to measure the copper oxide nanoparticles' UV-visible absorption spectrum. Throughout the reaction, sharp bands were seen up to 220 nm, which is a sign that CuO NPs were formed. Using scanning electron microscopy, the material's

morphology was examined. This demonstrates the spherical form of the artificial copper oxide nanoparticles (**Figure 2**). EDAX spectrum analysis of produced nanoparticles indicated the presence of copper (Cu) and oxygen (O). There are two peaks in the EDAX spectrum that are situated between 0.4 and 9 keV on the copper oxide nanoparticles. A second peak of O elements was found at 0.5 KeV, and the two peaks, which represented the purity of copper oxide nanoparticles, were situated at 0.9 and 8 KeV on the spectrum (**Figure 3**). Copper oxide nanoparticles were recorded with an EDAX spectrum that showed no peaks from other materials, showing that the particles were pure (**Figure 3**). The peaks of the XRD diffraction are reflected in the following indices: 36.4308, 43.3079, 50.4343, and 74.1074. (**Figure 4**). With the exception of the crystalline structure of CuO nanoparticles, which was discovered to be 75 nm, all diffraction peaks are indexed in accordance with the hexagonal phase of copper oxide nanoparticles (JCPDS NO. 36-1451). Analysis was done on the 400 to 4000 $cm^{-1}$ range of the copper oxide nanoparticles FTIR spectrum. With the help of the peak value in the infrared radiation area, the functional groups of the active components were determined by FT-IR measurement. 462.33 nm bands, which correspond to C–O alcohol, N=O Nitro group, C=CO Carbonyl, C–CL Alkaline Halide, and C-N Amine, were proven to represent copper oxide production (**Figure 5**).

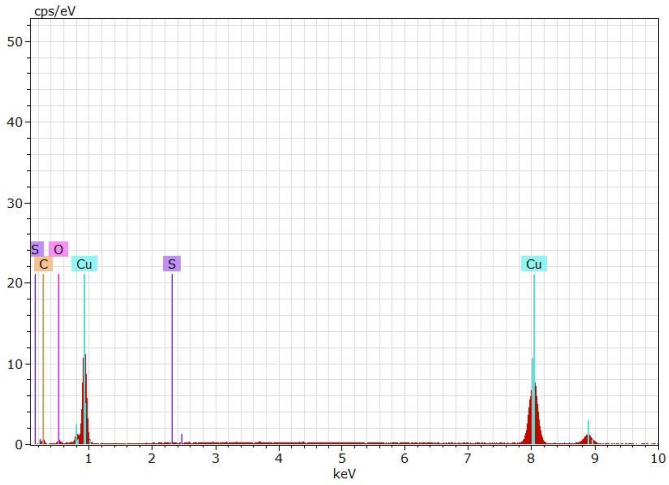


**Figure 3.** EDAX Image of CuO NPs.

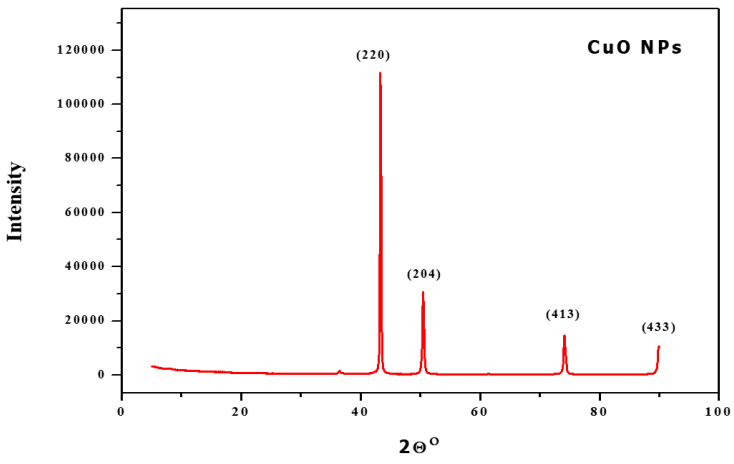

**Figure 4.** XRD Image of CuO NPs.

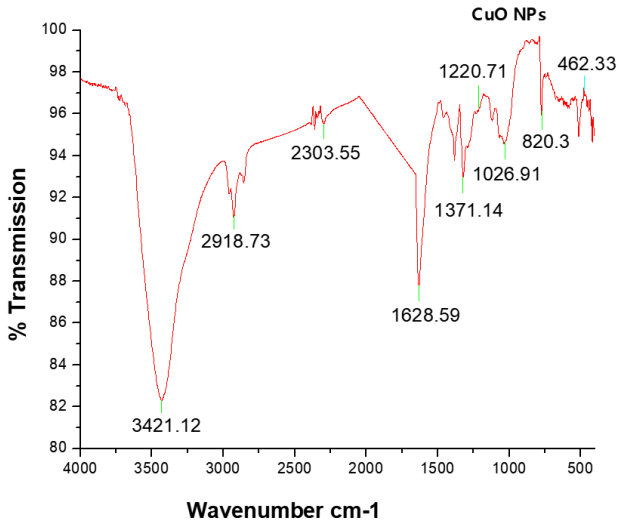


**Figure 5.** FT-IR Image of CuO NPs.

**Table 3** represents the condition factor of Koi carp raised on various meals. In every feed, the final condition factor is raised. **Table 4** displays the various feed usage and growth metrics. **Table 5** displays the results of the ANOVA (Analysis of Variance) for the growth parameters feed consumption, growth, gross growth efficiency, and net growth efficiency. Koi carp consumed more feed in feed V (8.03), which contained 150 mg of copper oxide nanoparticles, and less feed in feed IV (5.83), which contained 100 mg. The feed IV's feed conversion efficiency. Feed V (6.46) with 200 mg of copper oxide nanoparticles showed a greater growth rate. With feed V (6.46) containing 200 mg of copper oxide nanoparticles, the growth was greater. Analytical variance (ANOVA) demonstrates the significance of the growth (**Table 5**). Koi carp raised in feed VI have a greater % growth rate similar to growth (20.3). Koi carp showed improved absorption, metabolism, gross, and net growth efficiency in feed VI. The net and gross growth efficiency are considerable, as indicated by the analytical variance (ANOVA) (**Table 5**).

**Table 6** displays the haematological parameters of Koi carp. With an increase in the amount of copper oxide nanoparticles in the feed, Koi carp's red blood corpuscles, haemoglobin, hematocrit, and platelets gradually declined while their white blood corpuscles increased.

**Table 3.** Condition Factor (k) of Koi carp.

| Feeds | Initial | Final |
|---|---|---|
| I (Control) | 2.17 ± 0.26 | 2.73 ± 0.64 |
| II | 1.68 ± 0.21 | 2.38 ± 0.31 |
| III | 1.56 ± 0.15 | 3.44 ± 0.21 |
| IV | 1.82 ± 0.54 | 1.93 ± 0.63 |
| V | 1.73 ± 0.33 | 1.83 ± 0.43 |
| VI | 1.65 ± 0.14 | 2.35 ± 0.43 |

**Table 4.** Koi carp's feed utilization and Growth parameters in relation to the different quantities of copper oxide nanoparticles. Each value is the average (±SD) performance of five individuals in triplicates reared for 21 days.

| Parameters | Experimental feeds | | | | | |
|---|---|---|---|---|---|---|
| | I (Control) | II (25 mg) | III (50 mg) | IV (100 mg) | V (150 mg) | VI (200 mg) |
| Feed consumption(g/g live wt/21 days) | 7.9 ± 0.9 | 7.6 ± 0.4 | 6.7 ± 0.4 | 5.8 ± 1.0 | 8.03 ± 1.9 | 6.4 ± 0.5 |
| Feed conversion efficiency | 14.2 ± 1.82 | 13.4 ± 2.3 | 16.9 ± 0.88 | 21.09 ± 1.4 | 14.8 ± 2.6 | 20.3 ± 1.9 |
| Feed conversion ratio | 7.10 ± 8.97 | 6.71 ± 11.2 | 8..44 ± 4.5 | 10.54 ± 6.9 | 7.42 ± 13.04 | 10.16 ± 9.9 |
| Growth | 5.6 ± 0.12 | 5.5 ± 0.06 | 5.6 ± 0.06 | 5.5 ± 0.1 | 5.7 ± 0.06 | 6.03 ± 0.21 |
| Percentage growth | 71.1 ± 9.1 | 67.1 ± 11.1 | 84.4 ± .4 | 98.7 ± 3.4 | 74.2 ± 13.04 | 99.5 ± 9.9 |
| Relative growth rate | 2.8 ± 0.04 | 2.5 ± .0.34 | 2.8 ± 0.02 | 3.05 ± 0.3 | 2.9 ± 0.02 | 3.2 ± 0.15 |
| Assimilation (g/g live wt/21 days) | 2.3 ± 0.14 | 2.1 ± 0.21 | 2.1 ± 0.5 | 2.8 ± 0.4 | 1.9 ± 0.3 | 2.1 ± 0.2 |
| Metabolism (g/g live wt/21 days) | 3.3 ± 0.12 | 2.9 ± 0.5 | 3.6 ± 0.5 | 3.2 ± 0.2 | 3.8 ± 0.4 | 4.3 ± 0.5 |
| Gross growth efficiency (%) | 71.01 ± 10.9 | 67.1 ± 13.8 | 84.4 ± 5.4 | 73.4 ± 8.5 | 74.2 ± 15.9 | 61.24 ± 11.1 |
| Net growth efficiency (%) | 23.2 ± 3.7 | 23.2 ± 5.9 | 25 ± 9.1 | 21.4 ± 12.8 | 26.6 ± 7.9 | 26.5 ± 15.1 |

**Table 5.** Koi carp's growth parameters (feed consumption, growth, gross growth efficiency, and net growth efficiency) as determined by one-way ANOVA.

| Parameters | Sourceofvariation | Sumof squares | df | Mean squares | f | Sig |
|---|---|---|---|---|---|---|
| Feed Consumption | Between Group | 12.165 | 5 | 2.433 | 2.457 | 0.094NS |
| | Within Group | 11.882 | 12 | - | | |
| | Total | 24.047 | 17 | 0.990 | | |
| Growth | Between Group | 0.598 | 5 | 0.120 | 9.357 | 0.001S |
| | Within Group | 0.153 | 12 | - | | |
| | Total | 0.751 | 17 | 0.013 | | |
| Gross growth Efficiency | Between Group | 4052.816 | 5 | 810.563 | 6.148 | 0.005S |
| | Within Group | 1582.111 | 12 | - | | |
| | Total | 5634.927 | 17 | 131.843 | | |
| Net growthefficiency | Between Group | 6277.747 | 5 | 1255.549 | 5.576 | 0.007 NS |
| | Within Group | 2702.141 | 12 | - | | |
| | Total | 8979.887 | 17 | 225.178 | | |

**Table 6.** Koi carp's hematological parameters.

| Parameters | Control | T1 | T2 | T3 | T4 | T5 |
|---|---|---|---|---|---|---|
| WBC (Cells/cumm) | 3200 | 3600 | 4500 | 5100 | 5200 | 5400 |
| Hemoglobin (gm/dL) | 0.5 | 0.4 | 0.3 | 0.2 | 0.1 | 0.1 |
| RBC (Millions/cumm) | 0.18 | 0.1 | 0.1 | 0.1 | 0.01 | 0.01 |
| Haematocrit (%) | 1.1 | 1.2 | 0.9 | 0.6 | 0.3 | 0.2 |
| Platelets (Lakhs/cumm) | 70000 | 11000 | 6000 | 4000 | 3000 | 2000 |

## 4. Discussion

Copper oxide nanoparticles' UV-visible absorption spectra were observed between 200 and 300 nm in wavelength. Throughout the process, there were distinct bands visible up to 220 nm, which is a sign that CuO NPs were formed. According to

Nehru and Tharani [4], UV-Vis Spectroscopy was used to examine the optical characteristics. The UV-Vis spectra revealed that the CuO nanoparticles had a bandgap energy of about 6 eV. Using SEM examination, the surface morphology of the produced CuO NPs was examined. The synthetic copper oxide nanoparticles' spherical form is shown using scanning electron microscopy. According to Abbas Eslami et al. [5], the average size of CuO nanoparticles is between 50 and 70 nm, and they have suitable separation, spherical shape, and good homogeneity. The synthesized CuO nanoparticles showed signs of oxygen (O) and copper (Cu). The two peaks in the EDAX spectrum, which were observed on the copper oxide nanoparticles, are situated between 0.4 and 9 KeV. Two peaks on the spectrum, positioned at 0.9 and 8 KeV, respectively, denoted the purity of the copper oxide nanoparticles; a third peak, representing the O elements, was found at 0.5 KeV. The Energy-dispersive X-ray Analysis of the CuO nanoparticles and the data show that the nanoparticles are almost stoichiometric, according to Aparna et al. [6]. The index values for the XRD diffraction peaks are 36.4308, 43.3079, 50.4343, and 74.1074. The produced CuO nanoparticles' crystalline structure is shown by XRD examination. Every diffraction peak is indexed based on the copper oxide nanoparticles' hexagonal phase (JCPDS NO. 36-1451) According to GangarapuManjari et al. [7], the crystalline nature and monoclinic phase of the CuO nanoparticles (JCPDS-05-0661). Copper oxide nanoparticles' FTIR spectra were examined in the 400–4000 $cm^{-1}$ range. Bands 3421.12, 2918.73, 2303.55, 1628.59, 1371.14, 1220.71, and 1026.91 were linked to C–O alcohol, N=O Nitro group, C=CO Carbonyl, C–CL Alkaline Halide, and C–N Amine. Copper oxide formation was confirmed at 462.33 nm. Strong peaks were observed at 3441, 1633, 1046, and 1403 $cm^{-1}$, which correspond to OH, C=C, C–O, and aliphatic C–H stretching vibrations, respectively, in the FTIR measurements for the synthesised copper oxide NPs, according to Renuga et al. [8].

To evaluate the feed, the condition factor (K) of Koi carp was measured for comparative analysis. CuO nanoparticle-supplemented meals all showed an increase in the Koi carp's final condition factor. According to Srinivasan et al. [9], *Macrobrachiumrosenbergii* post-larvae fed 40g/$kg^{-1}$ of iron oxide nanoparticles in their diet showed an increase in condition factor. Koi carp's feed consumption and feed conversion efficiency were greater in feed V (8.03 and 20.31). Additionally, feed VI containing 100 mg of ZnO NPs in the feed increased the feed consumption and feed conversion efficiency of Koi carp, according to Rajan and Rohini [10]. Koi carp had a better feed conversion ratio in feed VI (96.03). According to Muralisankar et al. [3], *Macrobrachiumrosenbergii*, given zinc oxide, had a poorer feed conversion ratio than the control. Koi carp showed greater growth, percentage growth, and relative growth rate in feed V and feed VI. According to Davis et al. [11], the growth rate of *Penaeusvannamai* was progressively raised from lower concentrations to a greater concentration of diet treated with zinc. According to Muralisankar et al. [12], *Macrobrachiumrosenbergii* grew more when fed a diet supplemented with copper. The feed increased the assimilation and metabolism of koi carp. According to Sangeetha and Rajan [13], Koi carp assimilation was higher in feed IV, which contained 30 mg of iron oxide nanoparticles. Compared to the control, Koi's gross and net growth efficiency rose dramatically in feed VI, which

contained 200 mg of copper oxide nanoparticles. According to Soundhariya and Rajan [14], Koi carp's gross growth efficiency was better in feed VI that contained 100 mg of ZnO NPs. The feed with 80 mg of ZnO NPs, feed V, had a better net growth efficiency. According to Rajan and Rohini [15], *Cirrhinusmrigala* fed feed IV containing 15 mg of ZnO NPs in the feed had a better gross growth efficiency.

Fish species' health status is determined by haematological markers. From feed I to feed VI, the Koi carp's white blood corpuscles progressively rose while their red blood corpuscles, haemoglobin, hematocrit, and platelets steadily declined. According to Faiz [16], the haematological features of grass carp fed a diet supplemented with ZnO demonstrated a significant increase in MCHC and RBC values but a significant decrease in WBCs, Hb, HCT, MCV, and MCH values. According to Soundhariya and Rajan [14], as the amount of ZnO NPs increased from feed I to feed VI, the RBC and WBC count of Koi carp gradually increased.

## 5. Conclusion

The present study concludes that 200 mg/100g of copper oxide nanoparticles (CuO NPs) in the feed were suitable for the enhanced growth of Koi carp. Hence the ornamental fish keepers integrate copper in the feed of ornamental fishes.

**Author contributions:** Conceptualization, MRR and MDS; methodology, MRR; software, CK; validation, CK, MDS and MRR; formal analysis, MRR; investigation, MDS; resources, CK; data curation, CK; writing—original draft preparation, CK; writing—review and editing, MRR; visualization, MRR; supervision, MDS; project administration, MRR. All authors have read and agreed to the published version of the manuscript.

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**Conflict of interest:** The authors declare no conflict of interest.

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