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Impact Of Differential Quantities Of Magnesium Oxide Nanoparticles On Growth, Haematological And Biochemical Characteristics Of Common Carp *Cyprinus carpio*

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Abstract - Magnesium nanoparticles were synthesized and characterized by using a Scanning Electron Microscope (SEM), Energy Dispersive X-ray Spectroscopy (EDAX), X-ray diffraction (XRD), and Fourier Transforms Infrared Spectroscopy (FTIR). The objectives of the work are to study the impact of differential quantities of magnesium oxide nanoparticles on the growth, haematological and biochemical characteristics of common carp. Six feeds were prepared by using differential quantities of Magnesium Oxide nanoparticles such as 0(Feed1),10(Feed 2),20(Feed 3),30(Feed 4),40(Feed 5), and 50(Feed 6)mg with fish meal, groundnut oil cake, wheat flour and tapioca flour. Growth, haematological and biochemical parameters of common carp were estimated after 21 days of rearing. Morphological characteristics of magnesium nanoparticles were observed by using an SEM at the wavelength from 2. EDAX spectrum showed three peaks located at 0.5 KeV,1.3 KeV, and 2.5 KeV. In (XRD), the peaks are indexed as 39.60^0(111), 42.90^0(200), 62.86^0(202), 74.62^0(311), and 78.60^0(322). The FTIR spectrum was in the range of 400-4000^{-1}, and functional groups are Alcohols, ketones, alkynes, alkyl, and halides. Survival rate indicated that all the Common carp were active and healthy during the period of 21 days except Feed 4 and 5. Feed consumption and feed conversion efficiency of Common carp were higher in Feed 5. The Feed conversion ratio was good in Feed 6. The specific growth rate was higher in Feed 6. Assimilation and Metabolism were higher in Feed 3. Gross and net growth efficiency was higher in Feed 5. Haematological parameters increased with increased concentration of magnesium oxide nanoparticles. Total protein, carbohydrate and lipid in the muscle, gill, and liver of Common carp were higher in Feed 6.

Keywords: Magnesium oxide nanoparticles, growth, haematological, biochemical, common carp

Highlights

- Leaf-like magnesium oxide nanoparticles were synthesized by chemical method.
- Morphology, size, form, and functional group of magnesium oxide nanoparticles were characterized using UV-visible spectroscopy, Scanning Electron Microscope, X-ray diffraction, and Fourier Transforms Infrared Spectroscopy.
- Feed 6 containing 50mg of magnesium oxide nanoparticles enhances the growth, hematological and biochemical characteristics of Common carp.

1. Introduction

The fisheries and aquaculture industry can be revolutionized by using nanotechnology tools for rapid disease detection and targeted delivery of drugs, DNA vaccines and nutrients. Micronutrients play a very important role in maintaining the good health of animals including fish, and prawns (Davies et al, 1996). Nano forms of minerals such as copper, zinc, iron, manganese, silver and selenium are new tools to improve the quality and sustainability of aquaculture production. These mineral forms have been reported to improve the survival, growth performance, muscle composition, digestive enzymes, carcass minerals and non-specific immune responses in fish and prawns (Rather et al, 2011, Muralisankar et al,2014, Srinivasan et al,2016, Sarkheil et al,2016). As an essential mineral, magnesium is the second most abundant cation within the cell and it is the co-factor for numerous enzymatic and metabolic pathways of protein, carbohydrate and lipid (Vormann,2003). It plays an important role in anti-oxidation and enhances immunity (Roy et al, 2007). It has been reported that the dietary supplementation of magnesium reduced oxidative stress and free radical generation and improved the meat quality of fish (Cheng et al, 2005). Dietary magnesium deficiency adversely affects the growth and survival of fish and shrimps. (Zhang et al,2011, Tang and Lv,2014). Biochemical and haematological parameters are widely used as health indicators in ecotoxicological studies because these parameters react before the toxicants enter the body of fish. Fish blood is a suitable way to determine and diagnose the toxicity of metal and metal oxide nanoparticles (Sevcikova *et al, 2016*, Ates *et al,* 2016), and hematological analysis is excellent for assessing the stress condition of aquatic organisms (Bahmani *et al,* 2012). Common carp, *Cyprinus carpio* is a freshwater fish cultured in various parts of the world, including India due to its low cost of production, high muscle content and easy rearing. Despite its economic importance, this fish is referred to as an ideal experimental animal model for studying toxic levels of aquatic ecosystems. However, there is a dearth of information pertaining to Magnesium oxide Nanoparticles on the basic physiology and biochemistry of fish. Hence, the present study was conducted to understand the impact of differential quantities of magnesium oxide nanoparticles on the growth, haematological and biochemical characteristics of Common carp *Cyprinus carpio.*

II.Materials and methods

2.1 Synthesis of magnesium oxide nanoparticles:

The Synthesis of MgO nanoparticles is divided into various steps, such as mixing, stirring, filtering, drying calcinating the powder at 400^0C for 3h, the MgO is obtained in the form of nanoparticles. Initially 5.21g (0.2M). Magnesium Nitrate Hexahydrate was dissolved in 200 ml of distilled water. 0.8 g (0.2M) of NaOH was dissolved in 200ml of distilled water. Then 200 ml of NaOH solution is added in a solution of $(MgNO_2H_2O)_6$) drop-wise by using a pipette. After that, the solution was kept under magnetic stirring for 2h after stirring on the table at rest for 2h so that, the precipitation is formed at the bottom of the beaker. This precipitate was filtered and washed several times by using distilled and ethanol so as to get the final product. After centrifuge, the final product is kept at room temperature for drying. This dried powder is then crushed and made into a very fine powder by using a mortar and pestle. Finally, fine powder of MgO is calcinated at 400^0C for 3h for the removal of impurities present in the powder. Synthesized MgO that possesses high crystallinity with a particle size in the nanosized range.

2.2 Characterization of magnesium oxide nanoparticles

2.2.1 Scanning Electron Microscope (SEM)

SEM analysis is a powerful investigative tool which uses a focused beam of electrons to produce complex, high-magnification images of a sample's surface topography. The morphology of the magnesium oxide was investigated using a scanning electron microscope (SEM) (LEO 1455 VP).

2.2.2 Energy Dispersive X-ray Spectroscopy (EDAX)

A minute drop of nanoparticles solution was cast on aluminium foil and subsequently dried in the air before transferring it to the microscope. An energy-dispersive X-ray detection instrument (EDAX) (HORIBA 8121-H) was used to examine the elemental composition of the MgO_2 NPs.

2.2.3 X-ray Diffraction (XRD)

The structure and crystalline size of MgO nanoparticles were determined by XRD using an X'pert powder x-ray diffractometer with nickel –filter CuKα radiatons in the 20 range (λ=1.5418Å from an X-ray tube run at 40kV and 30mg.

$$\lambda = 2d \sin\theta$$

d = interplane spacing, λ= wavelength of x-ray.

2.2.4 Fourier Transform Infrared Spectroscopy (FTIR)

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

2.3 Collection and acclimation of common carp fish:

For the present work, Common carp Cyprinus carpio fingerlings (0.72+0.2g) were collected from a Pandian fish farm, Dindigul, 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 28^0C. During acclimation, fish were fed with trainee feed containing fish meal, groundnut oil cake, wheat flour and rice bran in the 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. After knowing the protein content by Micro- Kjeldahl 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 binding agents; supplevite mix was also added. Six feeds were prepared by using differential quantities of Magnesium Oxide nanoparticles such as 0(Feed1),10(Feed 2),20(Feed 3),30(Feed 4),40(Feed 5), and 50(Feed 6)mg with fish meal, groundnut oil cake, wheat flour and tapioca flour(Table 1).

2.5 Experimental design for growth studies:

For the present study uniform size of *Cyprinus carpio* (1±0.05g) were selected and then the fishes were introduced in the trough having a capacity of 15 litres. 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 4-5 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 with the least disturbance to the fish and dried at 95ºC. Approximately 70% of the water in the tank was replaced with tap water. The experiment was continued for 21 days. On the 21st day length and weight of the fish were measured in live condition. The fish were sacrificed to Muscle, Gill and Liver from all experiments for further analysis.

Feed utilization parameters such as condition factor(K)(Weatherly and Gill,1987), survival rate, feed consumption, feed conversion efficiency, feed conversion ratio, growth, specific growth rate, assimilation, metabolism, gross and net growth efficiency were estimated after 21 days. Blood samples collected from fish in each exposure group were subjected to complete blood profile analysis (CBC) viz., White Blood Cells (WBC), Red blood cells (RBC), polymorph, neutrophils, lymphocytes and eosinophils and platelets were counted by hemocytometer method (Stevens, 1997), haemoglobin (Hb) were determined by cyanomethemoglobin method (Richard Lee *et al.,* 1998). The microhematocrit method was used for the determination of haematocrit (Hct), Mean corpuscular haemoglobin (MCH) and Mean corpuscular volume (MCV). Mean corpuscular haemoglobin concentration (MCHC) was calculated by using a standard calculation method (Nelson and Morris, 1989). After the collection of blood, fish were sacrificed for the collection of gill, muscle, and liver. Then dissected samples were rinsed with 80% saline to remove blood clots, weighed, and stored in frozen (-20ºC) condition until further biochemical analysis. Total protein content was determined by using Lowry's method (Lowry *et al.,* 1951). Total carbohydrate content was estimated by the Anthrone method (Carrol *et al*., 1956). Total lipid content was determined by the Folch method (Folch *et al.,* 1957).

III Results and discussion

Scanning Electron Microscopy indicates that magnesium nanoparticles are a leaf-like structure (Fig 1). Luca Pasquini *et al* ., (2010) reported the morphology and size of nanoparticles using SEM and magnesium oxide nanoparticles were more aggregates. Sellik *et al.,* (2017) also reported that monocrystalline material and randomly aggregated nanoparticles were visible. EDAX Analysis showed that the elemental composition of magnesium is higher than oxygen (Fig 2). EDAX result revealed that magnesium oxide nanoparticles and the peaks are located between 2.5Kev and 0.5 Kev. The EDAX spectrum shows that the product was principally composed of Mg and O(Rajendran et al, 2018).

The phase structure of biosynthesized magnesium oxide nanoparticles is shown in Figure 3. $2\theta = 39.60^0(111), 2\theta = 42.90^0(200), 2\theta = 62.86^0(202), 2\theta = 74.62^0(311), 2\theta = 78.60^0(222)$. The XRD spectrum suggests that magnesium oxide was crystalline. The crystal sizes calculated using Scherrer's formula were about 10nm. (Fig.3). Carmen et al., (2012) also reported γ- MgO and the particle size was calculated using Scherrer's equation estimated at around 10 nm. In order to evaluate and identify the reaction product, XRD was used for the crystal size analysis and XRD diffraction peaks are indexed as to 111, 200,202, and 311 (Zeyob Camtaken *et al.,* 2017).

The FT-IR spectra show bands at 3308,2139, 1635, 1346, 419, and 407 cm^{-1} and are related to O-H Bond vibrations of the hydroxyl group, alkyne group, C-H bending to aromatic tertiary amine group and magnesium oxide (Fig.4). The spectra of magnesium oxide show bands at 3450, 2922, 1639, 1383, 1250, 810 and 674 cm-1 and the functional groups were related to alcohol, phenol, alkaline, and ketone (Vyshnav et al, 2023). The peaks observed below 800cm^{-1} confirmed the magnesium oxide (Mohammad Moslem Imani and Mohsen Safaei,2019)

The condition factor of common carp *Cyprinus carpio* reared in different feeds was indicated in Table 2. The final condition factor increased in all feeds. A comparable condition factor has also been observed in Koi carp fed with zinc oxide nanoparticles (Soundhariya and Rajan, 2021**).** The different feed utilization and growth parameters are presented in Table 3. The survival rate indicated that all the common carp were active and healthy during the period of 21 days except feed 2, 5 & 6 (90,80 & 90%) respectively. The feed consumption was higher in feed 6 (3.26±0.57) and lower in feed 1 (control) (1.33±0.12g/live wt /21 days). The feed consumption is significantly varied (Table 4). The feed conversion efficiency is higher in feed 6(0.18) and lower in feed 1 (0.1). Lee and Putnam (1973) reported an increase in the concentration of Magnesium oxide nanoparticles with an increase in feed consumption and feed conversion in Common carp (*Cyprinus carpio).* The feed conversion ratio is good in feed 6 (2.15) when compared to other feeds. Mukesh Mehta Ambani (2015) reported that the feed conversion ratio was higher in control when compared to different concentrations of prepared feed of *Macrobrachium rosenbergii.* Muralisankar *et al*., (2014) reported that the feed conversion ratio was higher in control and lower in zinc oxide fed of *Macrobrachium rosenbergii*. The growth (0.76) and specific growth rate (3.2) were higher in feed 6. Davis *et al*., (1996) reported that the specific growth rate was gradually increased in lower concentrations to higher concentrations of zinc-supplemented feed of *Penaeus vannamai*. The assimilation, metabolism, gross and net growth efficiency of common carp were higher in feed 6. Rajan et al (2023) reported that the assimilation, metabolism, gross and net growth efficiency of *Cirrhinus mrigala* is higher feed 4 containing

75 mg of magnesium oxide nanoparticles. The analytical variance of (ANOVA) the gross and net growth efficiency is significant (P>0.05) (Table 4).

Haematological parameters are very helpful in judgment of the health condition of fish species. The whole blood parameters of Common carp are gradually increased from Feed 1 to Feed 6 (Table 5). The complete blood count of Common carp progressively increased in dose-dependent magnesium oxide nanoparticles. Abdel-Tawwab *et al*.,(2007) also reported an increase in blood parameters when compared to control with a high concentration of selenium nanoparticles supplemented feed to African catfish, *Clarias gariepinus*. Rajan and Rohini (2021) reported that all the haematological parameters of *Cirrhinus mrigala* increased with increased incorporation of ZnO nanoparticles in the feed. The total protein, carbohydrate and lipid content in muscle, gill and liver of Common carp is higher in feed 6 containing 50mg of magnesium oxide nanoparticles when compared to other feeds (Table 6). Kevasasus et al (2011) reported the concentration-based increase and decrease of protein, lipids and carbohydrates. The Cu, Zn, Fe, Ca, Mg, Na and K, have improved the synthesis of protein in aquatic animals and the optimum concentration of Mg can improve the synthesis of protein in shrimps and fishes. Ashouri *et al*.,(2015) also reported that the selenium nanoparticles in the feed increased the protein, carbohydrates and lipid content of muscle, gill and liver on crucian carp *Carassius auratus*.

IV. Conclusion

The results of the present study conclude that feed 6 containing 50mg of magnesium oxide nanoparticles was good for the growth, haematological and biochemical parameters of common carp.

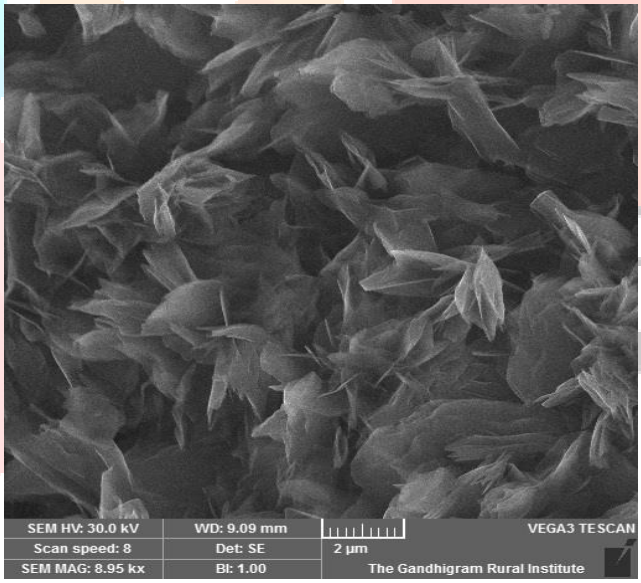

Figure 1: SEM image of magnesium oxide nanoparticles

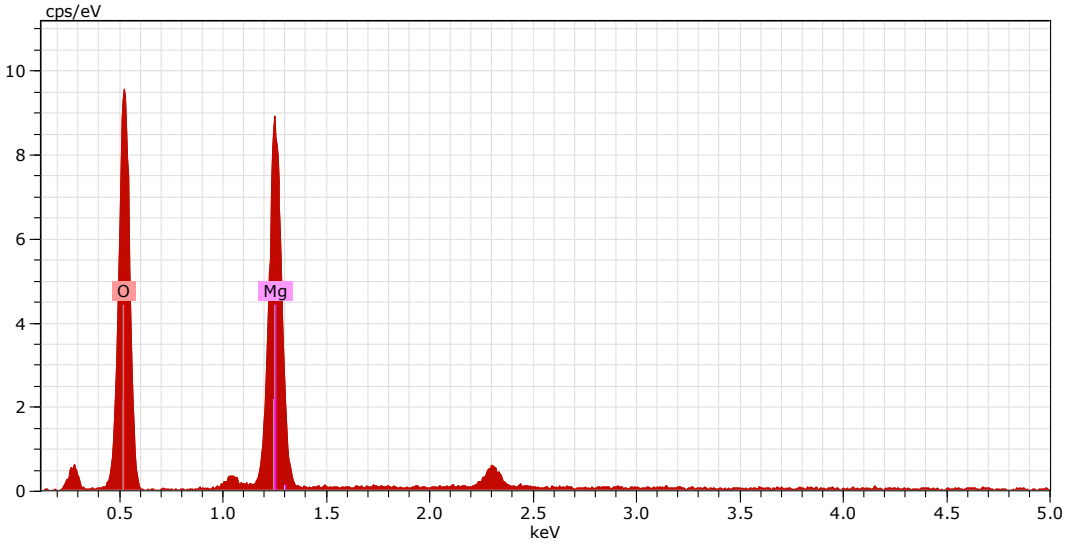

Figure 2: EDAX image of magnesium oxide nanoparticles

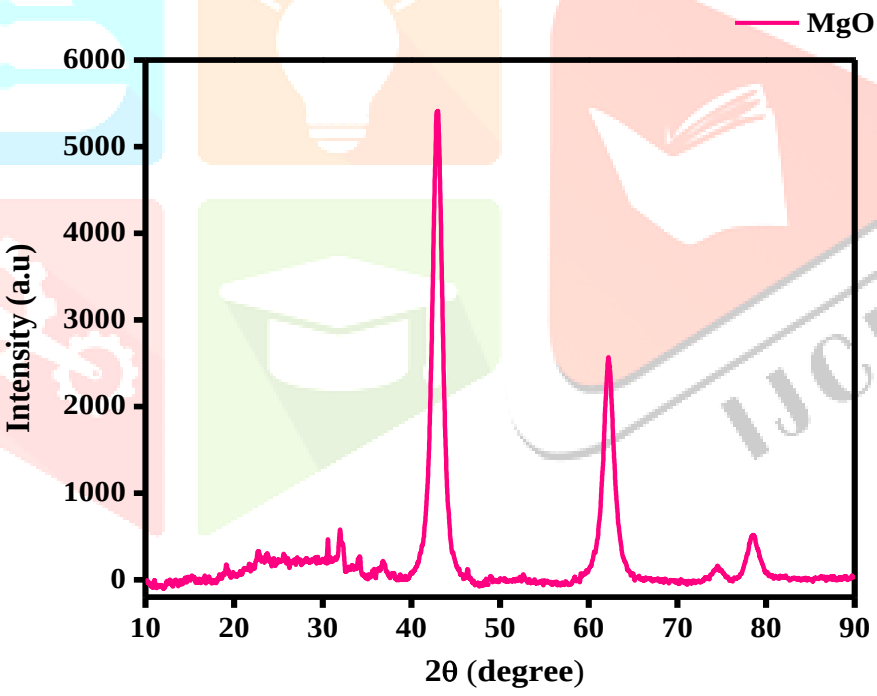

Figure 3: XRD image of magnesium oxide nanoparticles

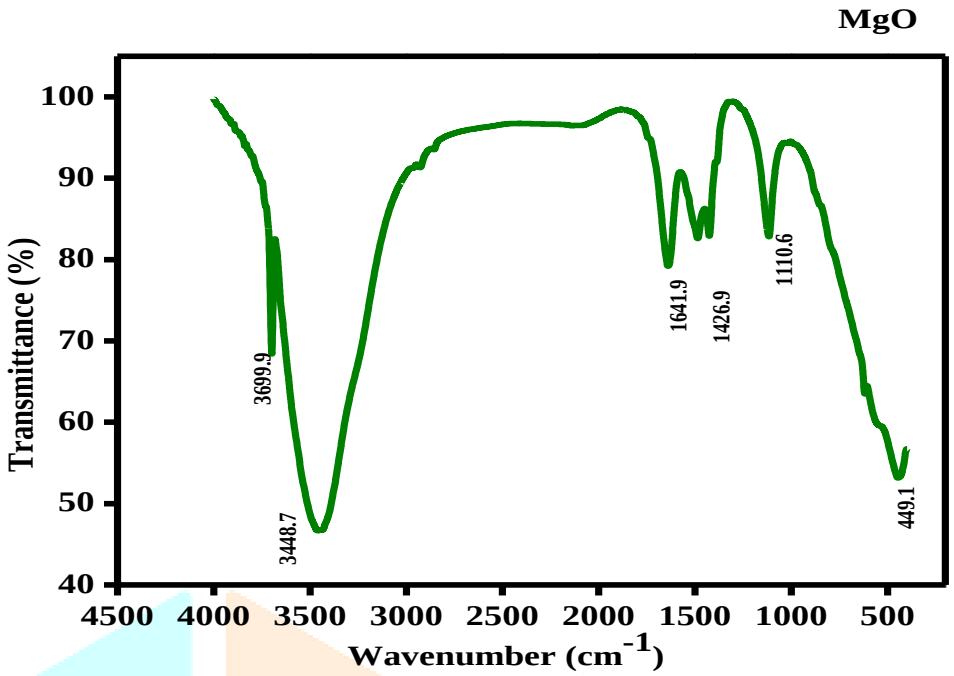

Figure 4: FT-IR image in magnesium oxide nanoparticles

Table 1: Composition of different feeds (g/100gm) of Common carp

	Experimental feeds					
Ingredients	**1(control)**	**2**	**3**	**4**	**5**	**6**
Fishmeal	36.2	36.2	36.2	36.2	36.2	36.2
GNOC	36.2	36.2	36.2	36.2	36.2	36.2
Wheat flour	8.7	8.7	8.7	8.7	8.7	8.7
Tapioca	8.7	8.7	8.7	8.7	8.7	8.7
Fish oil	2	2	2	2	2	2
Sunflower oil	2	2	2	2	2	2
Supplevite mix	4	4	4	4	4	4
Sodium chloride	1	1	1	1	1	1
Sodium benzoate	1	1	1	1	1	1
Magnesium oxide nanoparticles	0	10mg	20mg	30mg	40mg	50mg

GNOC- Groundnut Oil Cake

Table 2: Condition factor (k) of common carp

Feeds	Initial	Final
Ex. Feed 1 (control)	4.32±0.61	4.51±0.96
Ex. Feed 2 (10mg)	4.71±0.41	4.81±1.00
Ex. Feed 3(20mg)	5.18±0.30	5.42±0.59
Ex. Feed 4 (30mg)	4.53±0.66	4.81±0.63
Ex. Feed 5 (40mg)	5.00±0.82	5.23±0.94
Ex. Feed 6 (50mg)	5.49±0.57	5.63±0.63

Table 3: Feed utilization and growth parameters of common carp in relation to different quantity of magnesium oxide nanoparticles. each value is the average (± SD) performance of 5 individuals in triplicates reared for 21 days.

Parameters	Experimental feeds					
	1 (control)	2(10mg)	3(20mg)	4(30mg)	5 (40 mg)	6 (50mg)
Survival Rate (%)	100	90± 1.12	100	100	80±1.02	90±1.12
Feed Consumption (g/g live wt/21 days)	1.33±0.12[a]	1.5±0.2 [b]	2.83±0.89[c]	2.96±0.46[d]	3.1±0.92[e]	3.26±0.57[f]
Feed Conversion Efficiency	0.05±0.02	0.04±0.05	0.08±0.04	0.1±0.01	0.12±0.02	0.18±0.01
Feed Conservation Ratio	3.35±0.53	4.05±0.32	6.21±0.45	7.05±0.52	3.85±0.71	2.15±0.45
Growth(G) gm/gm live wt/21 days	0.25±0.05[a]	0.36±0.08[b]	0.48±0.04[c]	0.58±0.05[d]	0.68±0.05[e]	0.76±0.03[f]
Specific Growth Rate	1.52±0.12	2.02±0.16	0.53±0.21	0.9±0.10	1.8±0.23	3.2±0.31
Assimilation	1.2±0.16	0.87±0.25	1.2±0.38	1.45±0.34	1.53±0.61	1.6±0.25
Metabolism	1.16±0.20	1.2±0.3	1.18±0.52	1.3±0.12	1.53±0.48	2.3±0.24
Gross Growth Efficiency (%)	24.2±0.75[a]	26.1±2.7[b]	27.2±1.8[c]	29.6±2.6[d]	30.4±3.5[e]	44.03±1.7[f]
Net Growth Efficiency (%)	19.3±2.32[a]	18.5±2.54[b]	20.53±1.7[c]	24±1.44[d]	26.3±2.07[e]	28.6±1.73[f]

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

Table 4 ANOVA (Analysis of variance) of growth parameters (feed consumption, growth, gross growth efficiency, net growth efficiency) of Common carp

Parameters	Source	SS	Df	MS	F	PROB
Feed consumption	Columns	1.16256	03	0.24321	5.68	0.0121
	Errors	0.60056	10	0.05008		S
	Total	1.35743	15			
Growth	Columns	0.84065	04	0.10314	8.98	0.00087
	Errors	0.2253	11	0.12237		S
	Total	0.88665	17			
Gross growth efficiency	Columns	1329.78	03	448.78	7.55	0.0016
	Errors	748.87	10	50.765		S
	Total	24354.87	12			
Net growth efficiency	Columns	1652.44	03	325.98	8.78	0.0007
	Errors	354.82	16	33.897		S
	Total	1228.8	13			

Table 5: Haematological parameters of Common carp

Blood parameters	Feed 1 (Control)	Feed 2 (10 mg)	Feed 3 (20 mg)	Feed 4 (30 mg)	Feed 5 (40 mg)	Feed 6 (50 mg)
WBC (cells/cumm)	1,400	2,500	2,800	7,200	10,800	11,200
RBC (Millions/cumm)	0.04	0.06	0.02	0.08	0.09	0.3
Polymorph Neutrophils (%)	0.01	0.05	0.03	0.2	0.4	0.3
Lymphocytes (%)	0.4	0.6	0.1	0.06	0.7	0.4
Eosinophil (%)	0.3	0.1	0.04	0.07	0.02	0.2
Hemoglobin(gm/Di)	0.2	0.4	0.8	1.2	1.9	0.08
Hematocrit (%)	1.3	2.2	4.4	5.2	5.8	3.2
MCV	98	132	165	169	171	120
MCH	38	56	67	68	69	50
MCHC	21	42	41	40	38	20
Platelets (cells/cumm)	34,000	18,000	18,000	27,000	32,000	34,000

Table 6. Impact of differential quantities of MgO nanoparticles on total protein, carbohydrate and lipid in muscle, gill and liver of Common carp

Experimental Feeds		Protein (mg/g)	Carbohydrate (mg/g)	Lipid (mg/g)
1 (Control)	Muscle	0.46	1.20	1.00
	Gill	0.44	0.23	0.66
	Liver	0.04	0.20	0.32
2	Muscle	0.34	1.05	1.21
	Gill	0.23	0.33	0.48
	Liver	0.10	0.72	0.42
3	Muscle	1.20	1.40	1.32
	Gill	0.48	0.66	1.05
	Liver	0.15	1.05	0.66
4	Muscle	0.60	1.63	1.91
	Gill	0.52	0.86	1.23
	Liver	0.20	1.24	0.78
5	Muscle	1.4	1.74	2.00
	Gill	0.67	0.95	1.41
	Liver	0.07	1.30	0.87
6	Muscle	1.90	1.79	2.01
	Gill	1.45	0.99	1.41
	Liver	0.52	1.35	1.05

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

Data sharing is not applicable to this article as no datasets were generated or analyzed during this study

Competing Interest

Authors declare no conflict of interest

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Authorship

Both the author's works were equal, read, and approved in the final manuscript.

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