IMPACT OF DIFFERENT QUANTITY OF MANGANESE OXIDE NANOPARTICLES INCORPORATED FEED ON BIOCHEMICAL AND HAEMATOLOGICAL PARAMETERS OF COMMON CARP *CYPRINUS CARPIO VAR. COMMUNIS*

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Abstract

Manganese oxide nanoparticles were synthesized and characterized using UV-Visible Spectroscopy, Scanning Electron Microscope, Energy Dispersive X-ray Spectroscopy, X-Ray Diffraction and Fourier Transform Infrared Spectroscopy. Different quantities of Manganese oxide nanoparticles, such as 3, 6, 9, 12 and 15 mg, were incorporated in fish meal, groundnut oil cake, wheat flour and tapioca flour. Biochemical parameters such as p rotein, carbohydrate and lipid in muscle, gill and liver and haematological parameters such as total RBC,Hb, Hct, WBC and total Platelets of Common carp were estimated after 28 days. The UV- Visible adsorption spectr a demonstrate that MnO NPs were measured in the wavelength with in 300 to 400 nm. It exhibits strong adsorption peak at 335nm. SEM image showed that the Manganese oxide nanoparticles were observed at 8.90nm (2µm). EDAX spectrum showed two peaks and were located between 0.40 KeV and 6KeV.XRD image shows that the diffraction peak is indexed as 110, 310, 111, 101, 211 and 102. FT-IR of the Manganese oxide nanoparticles were observed at the wavelength range from 500-4000 cm¯1. All the biochemical and haematological parameters were higher in feed IV. From the results, it is concluded that feed IV was suitable for biochemical and haematological parameters of Common carp.

Keywords: Manganese oxide, Nanoparticles, feed, biochemical, haematological, common carp

Introduction

Rapid progress in nanotechnology and nanoscience in the past few years has changed a lot in the fields of agriculture and allied fields, including fisheries and aquaculture. It can offer new techniques for aquaculture, fish biotechnology, fish genetics, aquatic health and fish reproduction, etc. Nanotechnology tools like DNA nanovaccines, nanomaterials, nanosensors, smart drug delivery, and Gene delivery, etc. have promising to solve many puzzles related to animal health, production, reproduction, prevention and treatment of diseases(Haldar et al, 2020). Nanomaterials are substances with grain sizes in the order of a billionth of a meter that possess unique and beneficial chemical, physical, and mechanical properties. These properties can be exploited for a wide range of applications, including next-generation computer chips, high-energy density batteries, high-sensitivity sensors, longer-lasting medical implants, and more. Nanomaterials have their own unique beneficial properties(Gajanan and Tijare, 2018). Nanomaterials can be arranged into four materials-based categories such as carbon-based nanomaterials, inorganic-based nanomaterials, organic-based nanomaterials, and composite-based nanomaterials. Based on dimension, nanomaterials were classified as 0D, 1D, 2D and 3D nanomaterials(Jeevanandam et al., 2018). Among various metal oxide nanoparticles, manganese oxide has attracted considerable interest due to its potential applications in many fields. Manganese oxides, including MnO2, Mn3O4 and MnO4, are used in wastewater treatment, supercapacitors and rechargeable batteries. Manganese (Mn) is an essential micronutrient for growth, reproduction and prevention of skeletal abnormalities in terrestrial animals and fish(Arthur Anderson,1953). While the dietary requirement of Mn varies among different species, it plays a significant role in the functioning of the central nervous system(Swaminathan,1986). Fish can absorb Mn from both their feed and the aquatic environment, but dietary supplementation may still be necessary. Mn is also crucial for better survival, muscle composition, immune response, antioxidant defence, and stress tolerance in some fish and crustaceans(Asaikutti et al.,2016). The element functions as an enzyme activator, particularly those involved in the citric acid cycle, bone formation, mucopolysaccharide synthesis, red blood cell regeneration, carbohydrate metabolism, and the reproductive cycle (FAO,1987). Nanotechnology offers great potential for improving aquaculture by providing novel nanotools that can address these issues(Jayasankar, 2018). The common carp *Cyprinus carpio var. communis* is an important species in freshwater aquaculture, accounting for 10% of global production. Its feeding behaviour affects nutrient availability in the water column and can enhance photosynthesis and plankton production if the density is not excessive. However, excessive density can cause ecological

disruption at both community and ecosystem levels. The critical density of common carp depends on habitat and can strongly affect water quality, natural food resources, and fish growth. Common carp is a potential candidate for both monoculture and polyculture ponds, as it can switch to less preferred food when its preferred food is insufficient, and artificial feed can increase the critical density (Rahman, 2015). Few studies have investigated the effects of nanoparticles on fish; in this study, the common carp was used as the experimental model organism. Biochemical and Haematological studies furnish an index of physiological changes in fish, and the fish blood acts as an impressive tool for the detection of alterations in the tested organism(Adhikari et al., 2007). The work related to Manganese oxide nanoparticles incorporated feed on biochemical and haematological characteristics of Common carp is lacking. Hence, the present study was carried out.

Methodology

Synthesis of Manganese Oxide Nanoparticles by Co-precipitation Method

Manganese sulphate monohydrate (MnSo4.H2O) and Sodium hydroxide were used for the synthesis of manganese oxide nanoparticles (MnO) and were purchased from Spectrum reagents and chemicals pvt. Ltd, & Oxford Laboratory, India. All the reagents used for the synthesis of MnO nanoparticles were of analytical grade and used without further purification. All the glassware was washed, rinsed with deionised water, dried, and heat sterilized in a hot air oven. Manganese oxide nanoparticle is prepared by using 1.1M Manganese sulphate monohydrate in 100ml of distilled water. The solution is mixed and stirred for 1 hour using a magnetic stirrer and uncontaminated magnetic beads. Set the Magnetic stirrer at 60ºC. Then 0.1M of sodium hydroxide is added dropwise by using a pipette until the pH 8 is maintained. After one hour of stirring centrifuged three times with distilled water and ethanol. Dried the precipitate in a hot air oven at 100ºc for 2 hours. Kept the powder for calcination in a muffle furnace at 200ºC. The calcined product is ground using a mortar and pestle and subjected to further characterization by UV-Vis, SEM, EDAX, XRD and FT-IR(Fig. 1).

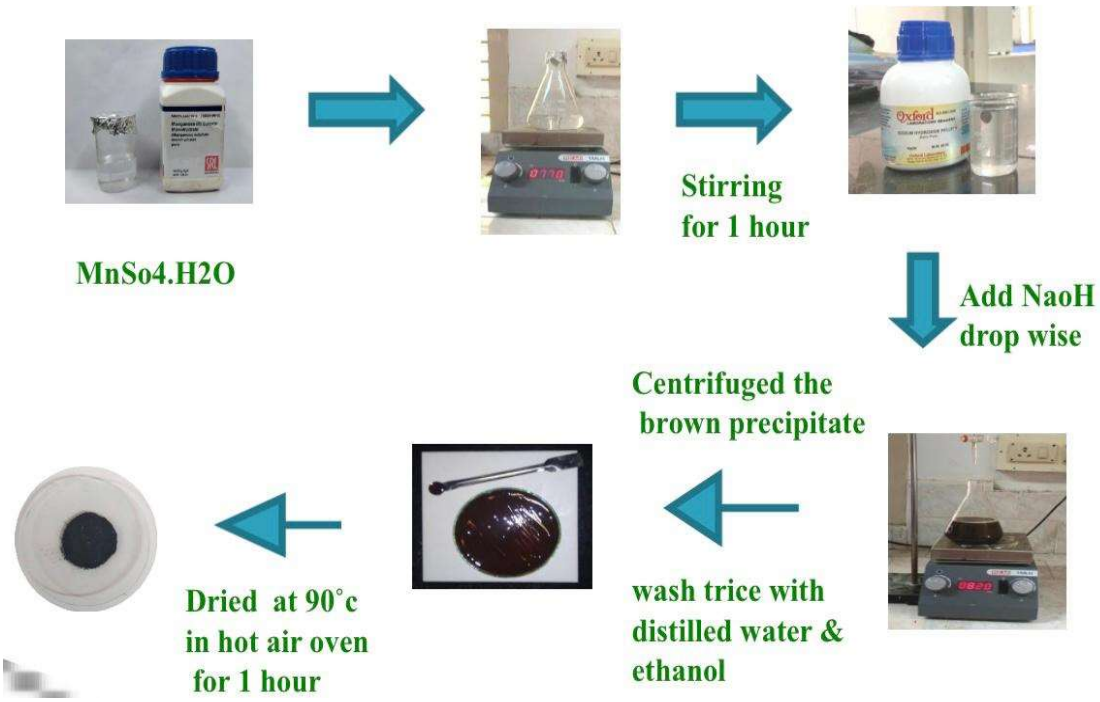

Fig.1 . Chemical Synthesis of Manganese Oxide Nanoparticles

Collection and Acclimation of Common Carp

For the present work, Common carp *(Cyprinus carpio var. communis)* fingerlings (1 ± 0.05g) were collected from K.V.R Fish farm, Palani, Tamil Nadu, India and transported to the laboratory in polythene bags filled with oxygenated water. Fishes were acclimated in concrete tanks for 15 days at 28°C. During acclimation period, fishes were fed ad-libitum with trainee feed containing fish meal, groundnut oil cake, wheat flour and rice bran in the form of dry pellets.

Selection of Feed Ingredients and Experimental Feed Preparation

Fish meal (Anchovy) and groundnut oil cake were used as protein sources; wheat flour and tapioca flour were used as carbohydrates sources; vegetable oil (Sunflower) and fish oil was used as lipid source and also as binding agents; Supplevite mix (Virbac Chelated Agrimin ® Forte) was used as source of vitamins and minerals; sodium chloride(NaCl), sodium benzoate (C_6H_5COONa) were used as preservatives. The components used for feed preparation were dried, powdered and sieved through a 425 micron sieve. After knowing the protein content (Table 1) by Micro-Kjeldhal method (Jayaraman, 1992), the feed was prepared by Pearson's Square method of ration formulation (Ali, 1980). The major ingredients (fish meal, groundnut oil cake, tapioca flour & wheat flour) were weighed and mixed thoroughly with 130-150 ml of distilled water. The mixed feed stuff was put in the autoclave for 30 minutes (at 121°C & 15 psi pressure) and cooled. After cooling, the minor ingredients i.e.. fish oil, sunflower oil, supplevite mix, sodium chloride, sodium benzoate and manganese oxide nanoparticles (3, 6, 9, 12,15 mg/100) were mixed with the feed, and it was extruded with the help of a pelletizer. The pellets were dried at room temperature in the shade (to avoid protein denaturation). The formulated feed was stored in air-tight containers at 20°C until use, to prevent microbial contamination (Table 2).

Table 1. Protein Level of Major Ingredients

S No	Ingredients	Protein (%)
1	Fish meal	58
2	Ground nut oil cake	44
3	Wheat flour	11
4	Tapioca	03

Table 2: Composition of Different Components in Experimental Feed (g/100gm) of Common Carp

S. No	Ingredients	Feed 1 (Control)	Feed 2	Feed 3	Feed 4	Feed 5	Feed 6
1	Fish meal	33.75	33.75	33.75	33.75	33.75	33.75
2	GNOC	33.75	33.75	33.75	33.75	33.75	33.75
3	Wheat flour	11.25	11.25	11.25	11.25	11.25	11.25
4	Tapioca	11.25	11.25	11.25	11.25	11.25	11.25
5	Fish oil	2	2	2	2	2	2
6	Sunflower oil	2	2	2	2	2	2
7	Supplevite-mix	1	1	1	1	1	1
8	Sodium chloride	1	1	1	1	1	1
9	Sodium benzoite	1	1	1	1	1	1
10	MnO nanoparticles	0	3mg	6mg	9mg	12mg	15mg

Characterization of Manganese Oxide Nanoparticles

Synthesized manganese oxide nanoparticles were characterized using UV-Visible spectroscopy(Thermoscientific GENESYS 180) ,Scanning Electron Microscope (VEGA 3 TESCAN type),Energy Dispersive X-ray Spectroscopy (BRUKER XFlash 6130), X-ray Diffraction(PANalytical XPert 3 Powder model) and Fourier Transform Infrared Spectroscopy (Jasco FT/IR-4700).

Experimental Design for Fish Growth Studies:

For the present study, uniform-sized Cyprinus carpio var. communis (1 ± 0.05g) were selected, and then the fish were introduced into the rectangular glass tank, having a capacity of 18 litres. Then the water in the tank was maintained at 15 litres. The initial length and weight of the fish were taken using a weighing machine and a ruler in live conditions without harming the fish. Ten fish were introduced into each tank. For each treatment, triplicate samples 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. Approximately 70% of the water in the tank was replaced with tap water. The experiment was continued for 28 days. On the 28th day length and weight of the fish were measured in live condition for the calculation of the condition of the fish.The condition factor (K) was calculated as per Weatherly and Gill (1987), for individual fish before and after the experiment.

Biochemical Characterics

Protein, carbohydrate and lipid in Gill, Muscle and Liver of Common carp

Quantities of protein were determined spectrophotometrically at 660nm based on Lowry's method(Lowry et al., 1951). The carbohydrate was determined by the Anthrone method (Carrol et al., 1961). Total Lipid was estimated by the Folch method (Folch et al., 1951).

Haematological Parameters:

Blood samples were collected from the fish after 28days, from the cardinal vein on the right side of the fish using a disposable insulin syringe fitted with a 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 (Hb), Haematocrit (Hct), were estimated after 28 days.

Results and Discussion

The UV- Visible absorption spectroscopy is widely used technique to examine the optical properties of the nanosi zed particle. The absorbance spectra of Manganese oxide nanoparticles were measured in wavelength within the wavelength range 300 to 400nm. It exhibits a strong absorption band at 335 nm, as shown in Figure 2. As the particle size decreases absorption wavelength will be shifted to shorter wavelengths, and the band gap increases for the nanosized particles. The absorption of ultraviolet and visible light involves the transition of electrons from the ground state to a higher energy state. This is the quantum confinement effect of nanoparticles. Haris Kumar et al. (2013) reported the sharp absorption peak at 339.60 nm from the UV-Vis analysis of MnO NPs synthesized by the co-precipitation method. Elsa Cherian et al., (2016) studied that the optical absorption spectra of manganese dioxide nanoparticles by UV-VIS spectrophotometer in the range of 250nm to 500 nm. An absorption peak at 340 nm indicates the .presence of manganese dioxide (Deogratius et al., 2013). Scanning electron microscopy shows that nanoparticles formed of clusters with distinct boundaries. Light and dark contrast in SEM images shows that the p articles are spherical in shape and possess depression on the surface in the middle as compared to their boundaries (Fig.3). The size of the particles lies in the 45-55 nm range. The surface morphology of the chemically synthesized MnO nanoparticles is investigated by using SEM analysis. Kamran et al. (2019) reported that the SEM images indicate the spherical-like shape of synthesized nanoparticles. The size of the synthesized manganese dioxide nanoparticles was found to be in the range of 40.5 - 70 nm (Elsa Cherian et al., 2016). The presence of oxygen (O) and Manganese (Mn) in synthesized nanoparticles were revealed by EDAX spectral analysis. The EDAX spectrum recorded on the manganese oxide nanoparticles is shown at two peaks located between 0.40 keV and 6.0 KeV. The two peaks indicating the purity of the manganese oxide nanoparticles were located on the spectrum at 0.62 KeV and 5.9 KeV, and another peak of the O element was located on at 0.46 KeV (Fig.4). The composition of elemental manganese was 38.99% as recorded in EDX analysis. EDX spectrum of manganese oxide nanoparticles clearly reveals the presence of two prominent peaks of manganese and oxygen present on the spectrum, thereby indicating the purity of the MnO nanoparticles. The XRD technique is used for structure and phase analysis of all compounds. The XRD diffraction peaks of MnO nanoparticles are indexed as 110, 310, 111, 101, 211and 102, which is represented in Fig.5. The clear and sharp diffraction peaks confirmed that the prepared compounds are pure with a high degree of crystallinity. Shayantani Chattarjee et al., (2017) characterized the green synthesised MnO NPs from *Brassica oleracea* (cabbage) leaf extract using XRD and reported 15.98% Manganese content. Saba Jamil et al. (2018)subjected the chemically synthesized MnO NPs to XRD analysis and reported that all the peaks are very sharp and indicate a crystalline nature of product and no extra peaks is present in the XRD pattern, which shows that synthesized product does not contain any impurities. X-ray powder diffraction (XRD) is a rapid analytical technique primarily used for phase identification of a given material and can provide information on unit cell dimensions. The FTIR spectrum of Manganese oxide nanoparticles was analyzed in the range of 500-4000 cm-1.(Fig.6). The FT-IR analysis was carried out to identify the functional groups of active components based on the peak value in the infrared region radiation. Manganese oxide formation was confirmed at 518 nm, bands have 3121, 1744, 1622, 1536, 1227, 1076, 848 and 512 were associated with alcohol, -lactose, conjugated alkene, nitro compound, carboxylic acid, amine, primary alcohol and halo compound, respectively. Ogunyemy et al. (2016)

that the Fourier transform infrared spectroscopy (FTIR) analysis of the freeze-dried powder of the reduced magnesium oxide and manganese dioxide nanoparticles was recorded under identical conditions in the range 400–4000 cm^{-1} at 4 cm^{-1} resolution using an FTIR spectrophotometer. Soundhariya *et al.* (2023) reported that the FT-IR spectrum of manganese oxide was measured at the wavelength range from 500-4000cm-1.

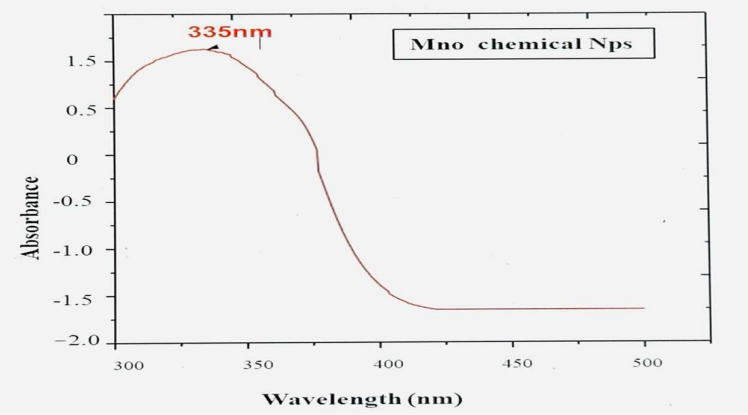

Fig 2. UV-Visible absorption spectrum of Manganese oxide nanoparticles

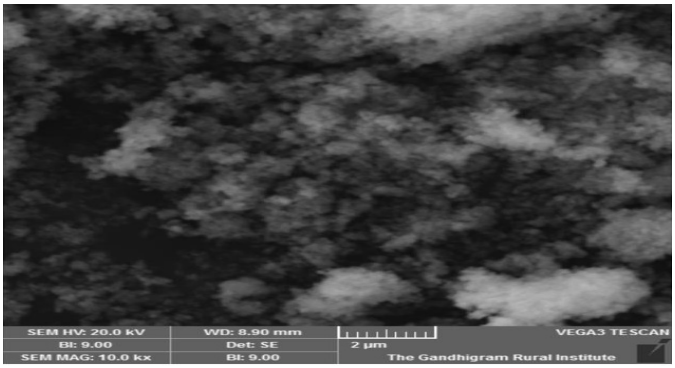

Fig 3. SEM image of manganese oxide nanoparticles

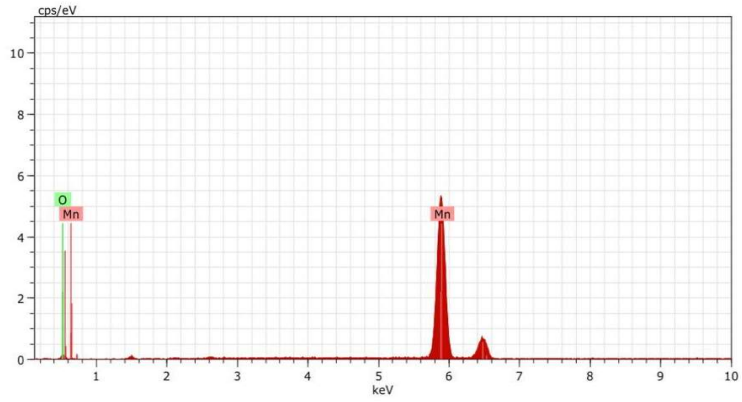

Fig. 4 EDAX spectrum of Manganese oxide nanoparticles

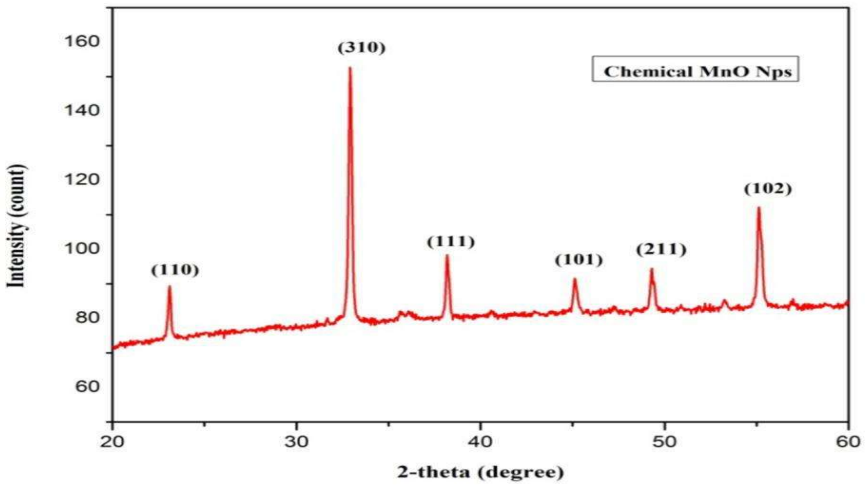

Fig.5 XRD spectrum of Manganese oxide nanoparticles

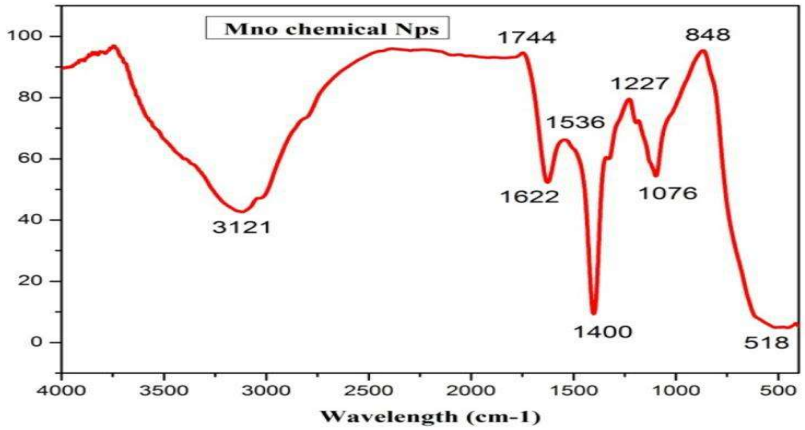

Fig.6 FT-IR spectrum of Manganese oxide nanoparticles

Total protein, carbohydrate and lipid content (mg/g) in muscle, gill and liver of common carp is higher in feed IV containing 9mg of manganese oxide nanoparticles when compared to other feeds.(Table. 4). Muthuswami Ruby Rajan et al. (2021) studied the effect of green-synthesized iron oxide nanoparticles on biochemical parameters of Zebra fish. Biochemical parameters such as carbohydrate, protein and lipid in muscle, gill and liver of common carp are higher in feed IV. Keerthika et al. (2017) reported that the iron oxide nanoparticles altered the biochemical parameters of *Labeo rohita.* In this present study, the higher level of total protein, carbohydrates, and lipids in the Muscles of Zebrafish in 40 mg/Kg-1 Fe2O3 nanoparticles-supplemented feed.

Table 4. Total protein, carbohydrate and lipid of common carp

Feeds	Tissues	Protein (mg/g)	Carbohydrate (mg/g)	Lipid (mg/g)
I (Control)	Muscle	0.331	1.245	0.27
	Gill	0.120	0.533	0.30
	Liver	0.012	0.588	0.20
II	Muscle	0.400	2.541	0.56
	Gill	0.282	0.660	0.52
	Liver	0.166	0.307	0.33
III	Muscle	0.065	2.620	0.75
	Gill	0.065	1.1060	0.60
	Liver	0.073	0.806	0.56
IV	Muscle	0.567	3.590	0.95
	Gill	0.329	1.228	0.85
	Liver	0.288	1.228	0.71
V	Muscle	0.184	1.105	0.59
	Gill	0.280	0.762	0.70
	Liver	0.061	0.441	0.53
VI	Muscle	0.130	1.576	0.78
	Gill	0.260	0.735	0.79
	Liver	0.067	0.107	0.64

Haematological parameters of Common carp are presented in Table 5. The RBC, WBC, Haemoglobin, RBC, Haematocrit (Hct) and Platelets are higher in Feed IV containing 9mg of manganese oxide nanoparticles when compared to other feeds. Haematological analysis of blood parameters is considered as physiological indicator of the whole body and therefore is important in diagnosing the structural and functional status of fish exposed to nanoparticles. (Rajendra Shejwal et al.,2014). All the haematological parameters are higher in feed IV. Haematological parameters are very helpful in the judgment of the health condition of fish species. The WBC count of zebrafish is gradually decreased as the quantity of ZnO NPs increases from feed I to feed VI. The platelets are increased with the increasing quantity of Zinc oxide nanoparticles in the feed (Muthuswami Ruby Rajan and Kamaraj Ramana Devi (2022)

Table 5. Haematological parameters of Common carp

Blood Parameters	FEED I	FEED II	FEED III	FEED VI	FEED V	FEED VI
RBC (millions/cumm)	0.02	0.02	0.02	0.06	0.03	0.03
Hemoglobin (gm/dl)	0.3	0.5	0.4	0.7	0.4	0.2
Haematocrit (PCV) (%)	0.1	0.3	0.2	0.6	0.3	0.2
WBC count (Cells/cumm)	1,900	5,300	2,400	6,600	2,000	1,300
Platelets count (Lakhs/cumm0	14,000	25,000	18,000	34,000	26,000	14,000

RBC - Red blood corpuscle WBC - White blood corpuscle

CONCLUSION

The present study concluded that the Feed IV containing 9mg of manganese oxide nanoparticles is suitable for the biochemical and haematological characteristics of Common carp.

CONFLICT OF INTEREST

There was no conflict of Interest

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