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Impact of differential quantity of iron oxide nanoparticles incorporated feed on growth, biochemical and haematological characteristics of Mrigal *Cirrhinus mrigala*

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Abstract

The present study deals with the impact of differential quantity of iron oxide nanoparticles incorporated feed on the growth, biochemical and haematological characteristics of Mrigal. Iron oxide nanoparticles were synthesised and characterized by using 5 UV-Vis spectroscopy(UV-Vis) , Scanning Electron Microscopy (SEM), Energy Dispersive X-Ray Spectroscopy (EDAX), X-Ray Diffraction (XRD), Fourier Transform Infrared Spectroscopy (FT-IR) andVibrating Sample Magnetometer (VSM). Six experimental feeds were prepared by using a fish meal, groundnut oil cake, wheat flour, tapioca flour and differential quantities of iron oxide nanoparticles such as 0, 10, 20, 30, 40, and 50 mg/100g. Feed utilization, biochemical and haematological characteristics of Mrigal were estimated after 21 days. UV-VIS Image spectra show that iron oxide nanoparticles were measured at a wavelength of "328 nm". SEM image shows the morphological formation of iron oxide nanoparticles and was observed at the wavelength of 1µm.EDAX spectrum recorded on the iron oxide nanoparticles is shown as two peaks located between 0.2KeV and 6.4KeV. XRD results were obtained for the crystal nature of iron oxide nanoparticles and the average size is 20 nm. FT-IR spectrum of iron oxide nanoparticles was analyzed in the range from 4000-500 cm-1.VSM results revealed that saturation magnetization (MS) is 1.019 emu/gm present in iron oxide nanoparticles. The feed consumption, feed conversion efficiency, growth, and relative growth rate of Mrigal were higher in Ex. Feed III. The gross and net growth efficiency of Mrigal were higher in Ex. Feed I. All the blood parameters were gradually increased from Feed I to VI. The total protein, carbohydrate and lipids of Mrigal's muscle, gill and liver of Mrigal is higher in Feed VI containing 50mg of iron oxide nanoparticles.

Keywords: Iron Oxide Nanoparticles; Feed; Growth; Biochemical; Haematology; Mrigal

1. Introduction

Among nanoparticles, iron oxide nanoparticles are gaining more attention in recent days. Iron oxide nanoparticles are highly marked by scientists due to their high biocompatibility, chemical stability and magnetic behaviours [1]. Iron (Fe) plays a vital role in physiological processes such as oxygen transport, cellular respiration, and the lipid oxidation reaction of organs and tissues of vertebrates including fish[2]. Iron is readily absorbed through the gastrointestinal tract, gills, fins, and skin of fish and crustaceans. In general, inorganic sources of iron are more readily absorbed than organic sources; ferrous iron (Fe++) is more available for absorption than ferric iron (Fe3+). Iron deficiency causes immune suppression, growth depression, changes in haematological parameters and microcytic anaemia in some freshwater fishes [3,4]. Mrigal is one of the Indian major carps which are an integral part of aquaculture and an important component of sustainable food security in India. Supplementary feed is the most critical input, judicious feed management enhances product performance and reduces product cost. The nanoparticulate diets increased the cholesterol and triglyceride levels more than the other treatments with the highest level observed in the iron oxide

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treatment [5]. Several studies have been carried out to evaluate the effect of feeding frequency on growth, survival, feed, intake, body composition etc [6]. Present study was designed to synthesise, characterization of Iron oxide nanoparticles and to assess the effect of dietary iron oxide nanoparticles on the growth, biochemical characteristics and haematological parameters of Mrigal *Cirrhinus mrigala.*

2. Materials and Methods

For growth studies, Mrigal fingerlings (1.575±0.30g) were collected from K.V.K Fish Farm, Palani, Tamil Nādu, India and transported to the laboratory and acclimated in round plastic troughs for a period of 15 days at 28±2° C. During acclimation, fish were fed with trainee feed containing fishmeal, groundnut oil cake, wheat flour and rice bran in the form of dry pellets.

2.1. Synthesis of Iron Oxide Nanoparticles

The co-precipitation method was used for the synthesis of iron oxide nanoparticles. The aqueous solution of $FeCl_2$ and $FeCl_3$ has prepared by 1:2 ratio and NaOH (0.1N) with constant stirring and within 30 minutes. The solution gets brownish-yellow colour. The pH of the solution was 1. The aqueous solution stirred within 30 minutes a visible colour change was observed. The yellowish-brown colour aqueous solution turned into a dark brown precipitate and the pH was adjusted to 12. After the precipitation, centrifuged at 500 rpm within 3 minutes, added ethanol in trace volume, collects the iron oxide dried nanoparticles and allow to air dry. Finally, iron oxide nanoparticles (Fe_3O_4) are formed.

2.2. Characterization of Iron Oxide Nanoparticles

The iron oxide nanoparticles were characterized using UV-Visible spectroscopy, scanning electron microscope (SEM) (LEO1445VP), energy dispersive X-ray detection instrument (HORIBA 8121-H), X-ray diffractometer, Fourier Transform Infrared Spectroscopy and Vibrating sample magnetometer.

2.3. Selection of Feed Ingredients and Experimental Feed Preparation:

The raw materials for feed preparation were selected based on their ability to supply nutrients such as proteins, carbohydrates and lipids at the low cast. After knowing the protein content (Table 1) by Micro – Kjeldhal method [7], the feed was prepared by Pearson's Square method of ration formulation [8]. Fish meal 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 were used as lipid sources and also as binding agents; Supplevite mix (Virbac Chelated Agrimin® Forte) was used as a source of vitamins and minerals; sodium chloride, sodium benzoate (C6H5COONa) was used as preservatives. The components used for feed preparation were dried, powdered and sieved through a 425-micron sieve. 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 feedstuff 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, suppletive mix, sodium chloride (NaCl), sodium benzoate (C6H5COONa) and iron oxide nanoparticles (10, 20, 30, 40,50mg/g) were mixed with the feed and it was extruded with the help of pelletizer. The pellets were dried at room temperature in shade (to avoid protein denaturation). The formulated feed was stored in airtight containers at 20°C until use, to prevent microbial contamination (Table 2).

Table 1 The protein level of major ingredients

Sr. No	Ingredients	Percentage of Protein
1	Fishmeal	58
2	Groundnut oil cake	44
3	Wheat flour	11
4	Tapioca	03

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

Sr. No	Ingredients	Experimental Feeds					
		I (Control)	II	III	IV	V	VI
1	Fishmeal	33.75	33.75	33.75	33.75	33.75	33.75
2	Groundnut oil cake	33.75	33.75	33.75	33.75	33.75	33.75
3	Wheat flour	11.2	11.2	11.2	11.2	11.2	11.2
4	Tapioca	11.2	11.2	11.2	11.2	11.2	11.2
5	Fish oil	2	2	2	2	2	2
6	Sunflower oil	2	2	2	2	2	2
7	Supplevite mix	2	2	2	2	2	2
8	Sodium chloride	2	2	2	2	2	2
9	Sodium benzoate	2	2	2	2	2	2
10	Iron oxide nanoparticles	0	10 mg	20 mg	30 mg	40 mg	50 g

2.4. Experimental design for fish growth studies:

For the present study uniform weight of Mrigal *Cirrhinus mrigala* (1.575 ± 0.30g) was selected and then the fishes were introduced in the rectangular plastic trough (45 cm Lx30 cm Bx15 cm H) having a capacity of 15 litres. Then water in the trough was maintained at 10 litres. The initial length and weight of the fish were taken using a weighing machine and ruler in live conditions without harming the fish. Five fishes were introduced in each trough and for each treatment triplicates (6 X 3 =18) 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 and 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˚C in a hot air oven. Approximately 70% of the water in the tank was replaced with tap water. The experiment was continued for 21 days. On the 21st day, the length and weight of the fish were measured in live condition for the calculation of growth parameters, and collected muscle, gill, and liver was from all treatments for estimation of biochemical parameters such as protein [9], carbohydrate[10] and lipid[11]. For the haematological study blood samples were collected from fish after 21 days from the cardinal vein on the right side of the fish using the disposable insulin syringe fitted with the fine needle, without harming the fish. The syringe and needle were moistened with EDTA (an Anticoagulant). The collected blood was then transferred into an Eppendorf tube containing 0.1 N EDTA. Complete blood parameters such as RBC, WBC, Platelet count, Hemoglobin (Hb), and Haematocrit (Hct) were estimated.

3. Results

3.1. Characterization of Iron oxide nanoparticles

The absorbance spectra of Fe_3O_4 nanoparticles were measured in wavelength within the range of 200-800 nm. The sharp bands were observed close to “328 nm” throughout the reaction which indicated the formation of irons (Figure 1). Scanning Electron Microscopy indicated that nanoparticles formed as agglomerated because of the adhesive nature of distorted irregular cluster appearance as shown in figure 2 and due to the denaturation and aggregation during the sputtering process. EDAX spectrum recorded on the iron oxide nanoparticles is shown as two peaks located between 0.2KeV and 6.4KeV those directly related to the iron and characterized lines K. The maximum peak located on the spectrum at 6.4KeV clearly comes from iron. The second peak located on the spectrum at 0.2KeV clearly indicates oxygen (Figure 3). The XRD technique is used for all compounds' structure and phase analysis. The XRD diffraction peaks of FeO nanoparticles are indexed as 2702,2915,3223,3033,3065,2865 which is represented in Figure 4. The clear and sharp diffraction peaks confirmed that the prepared compounds are pure with a high degree of crystallinity. The FTIR spectrum of iron oxide nanoparticles were analyzed in the range of 4000-500 cm-1The FT-IR analysis was carried out for identifying the functional groups of active components based on the peak value in the region infrared radiation. Iron oxide formation was confirmed with bands 3364,2914,1626,1245,859,688 and 491, which were associated with

N-H Aliphatic primary amine, C-H Alkane, C-C Alkene, C-O Alkyl aryl ether, C=C Alkene, C-I Halo compound, C-I Halogen compound (Figure 5). The magnetic properties of synthesized nanoparticles in the presence of a magnetic field were measured using a vibrating sample magnetometer. Figure 6 shows that the saturation magnetization (MS) is 1.019 emu/gm.

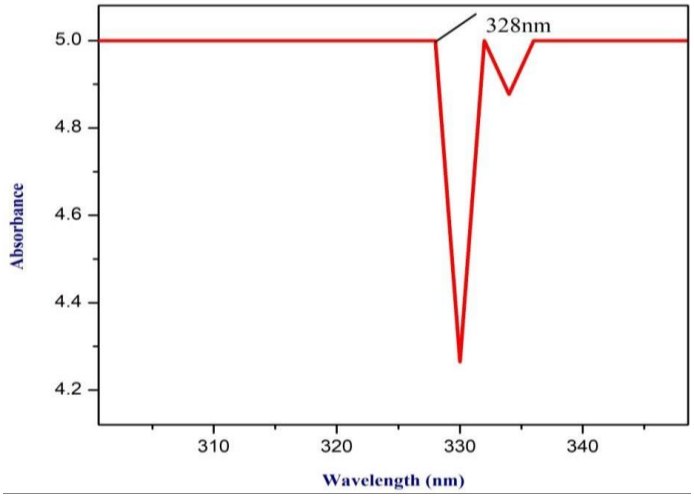

Figure 1 UV-Vis Image of Iron Oxide Nanoparticles

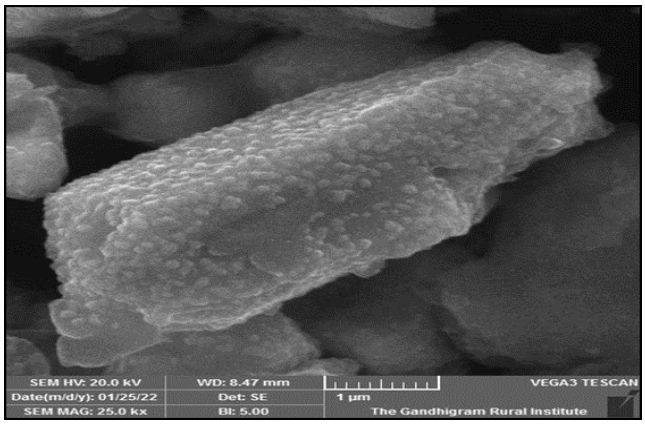

Figure 2 SEM Image of Iron Oxide Nanoparticles

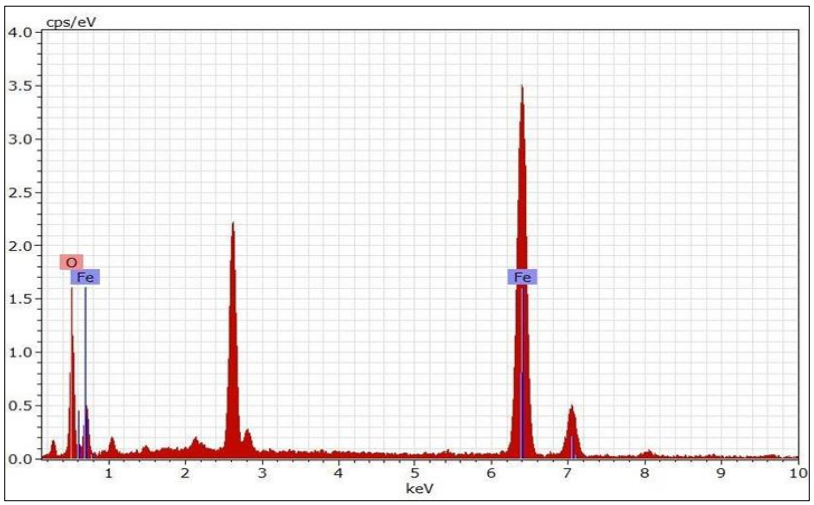

Figure 3 EDAX Image of Iron Oxide Nanoparticles

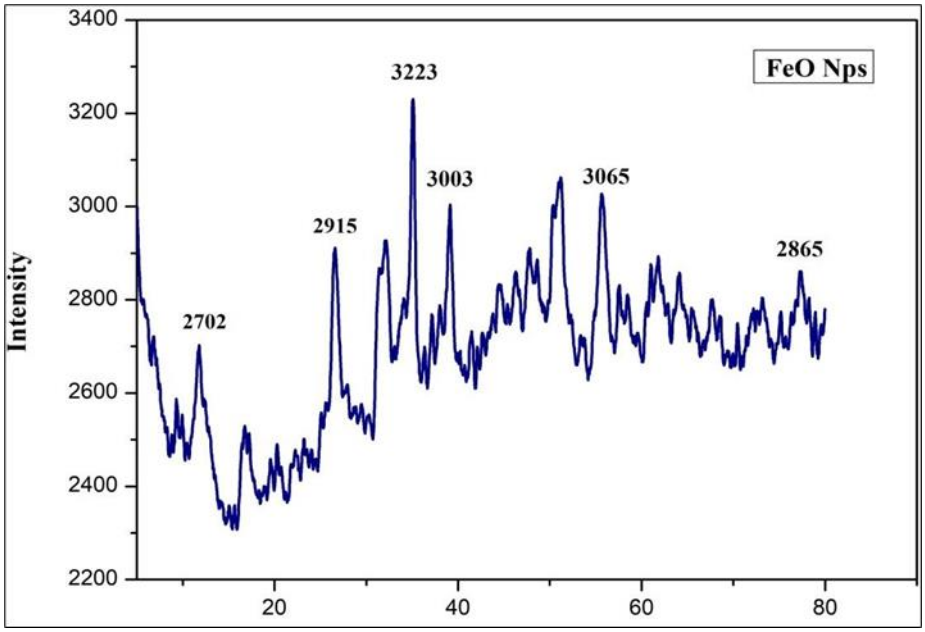

Figure 4 XRD Image of Iron Oxide Nanoparticles

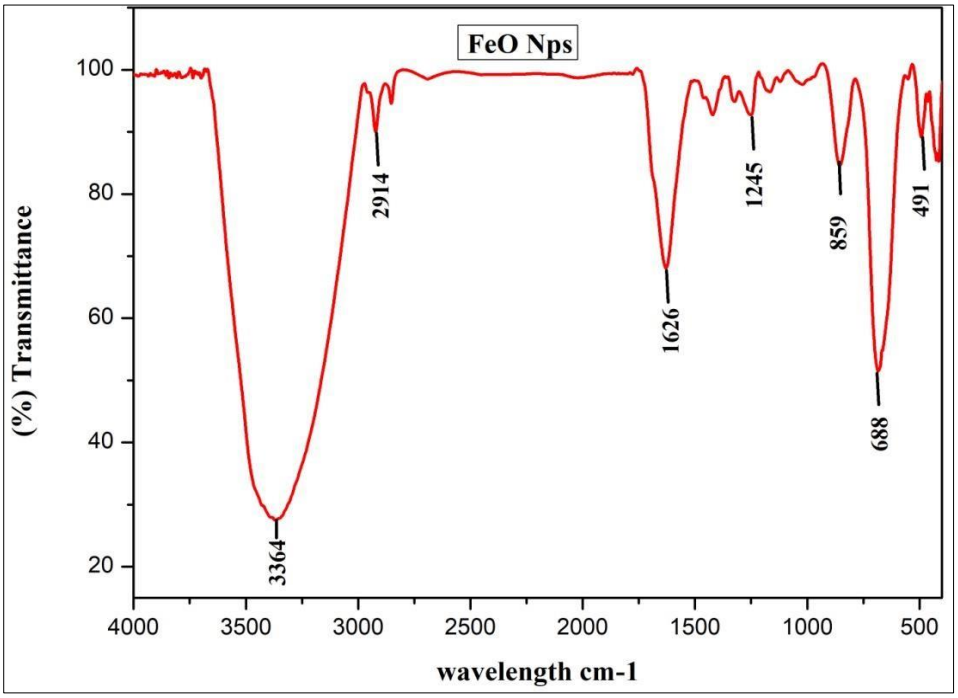

Figure 5 FT-IR Image of Iron Oxide Nanoparticles

The condition factor of Mrigal *Cirrhinus mrigala* reared in different feeds was indicated in Table 3. The final condition factor is decreased in all the feeds. Feed utilization parameters were presented in Table 4. The feed consumption was higher in feed III containing 20 mg of Iron oxide nanoparticles and lower in feed VI containing 50 mg of Iron oxide nanoparticles. The analytical variance of (ANOVA) the feed consumption is significant (P >0.05) (Table 5). The feed conversion efficiency is higher in feed III. The feed conversion ratio is best in feeds II and III. The growth of mrigal is higher in feed III (1.73) containing 20 mg of Iron oxide nanoparticles and lower in feed V. The analytical variance (ANOVA) shows that the growth is significant (P>0.05). Like growth, the percentage growth rate and relative growth rate of Mrigal were higher in feed III. The assimilation and metabolism were good in feed III. Among the treatments the gross and net growth efficiency of mrigal reared in feed III was higher. The analytical variance (ANOVA) shows that the gross and net growth efficiency is significant (P>0.05).

Table 3 Condition Factor (K) of Mrigal

Feeds	Initial	Final
I (Control)	3.42±0.26	3.14±0.10
II	3.01±0.46	3.34±1.03
III	3.19±0.70	2.62±0.74
IV	3.70±1.02	3.19±0.79
V	3.04±0.56	2.81±0.43
VI	2.56±0.29	2.44±0.31

Table 4 Feed utilization and Growth parameters of Mrigal in relation to the different quantities of Iron oxide nanoparticles. Each value is the average (± SD) performance of five individuals in triplicates reared for 21 days.

Parameters	Experimental Feeds					
	I (Control)	II (10 mg)	III (20 mg)	IV (30 mg)	V (40 mg)	VI (50 mg)
Feed consumption (g/g live wt/21 days)	5.82±2.0[a]	3.88±1.3[b]	5.49±2.3[c]	2.73±1.0[d]	3.99±1.52[e]	3.43±0.4[f]
Feed conversion Efficiency	0.33±0.22	1.63±0.39	1.67±1.26	0.78±040	0.11±0.06	0.16±0.80
Feed conversion Ratio	15.6±9.5	2.66±0.5	3.84±2.92	5.91±3.34	35.8±21.7	23.6±13.2
Growth	0.43±0.15[a]	1.43±0.25[b]	1.73±0.60[c]	0.5±0.1[d]	0.13±0.05[e]	0.16±0.05[f]
Percentage Growth	18.4±10.1	60.7±6.8	62.8±25.5	24.02±5.09	5.29±2.37	6.30±2.27
RelativeGrowth	0.21±0.07	0.71 ±0.12	0.86± 0.30	0.25± 0.05	0.06 ±0.02	0.08 ±0.02
Assimilation (g/g live wt/21 days)	0.93±0.31	0.84±0.26	0.87±0.03	0.45±0.14	0.72±0.51	0.61±0.35
Metabolism (g/g live wt/21days)	0.5±0.45	0.5±0.17	0.96±0.72	0.15±0.12	0.62±0.51	0.45±0.36
Gross Growth Efficiency (%)	33.5±22.0[a]	11.77±106.9[b]	16.73±126.5[c]	7.80±40.14[d]	11.19±6.42[e]	16.35±8.04[f]
Net Growth Efficiency (%)	55.8±25.1[a]	28.4±41.2[b]	22.89±122.5[c]	12.23±67.3[d]	27.33±23.4[e]	41.6±41.14[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 5 ANOVA (Analysis of Variance) of Growth Parameters (Feed Consumption, Growth, Gross Growth Efficiency, Net Growth Efficiency) of Mrigal

Parameters	Source of Variation	Sum of Squares	DF	Mean Squares	F	SIG
Feed Consumption	Between Groups Within Groups Total	11138.461 .005 11138.466	18 1 19	618.803 .005	17.451	0.02 S
Growth	Between Groups Within Groups Total	117905.273 .020 117905.293	18 1 19	6550.293 .020	26.221	0.01 S
Gross Growth Efficiency	Between Groups Within Groups Total	240824.692 245 240824.937	18 1 19	13379.150 .245	82.832	0.03 S
Net GrowthEfficiency	Between Groups Within Groups Total	60086.849 .020 60086.869	18 1 19	3338.158 .020	736.969	0.02 S

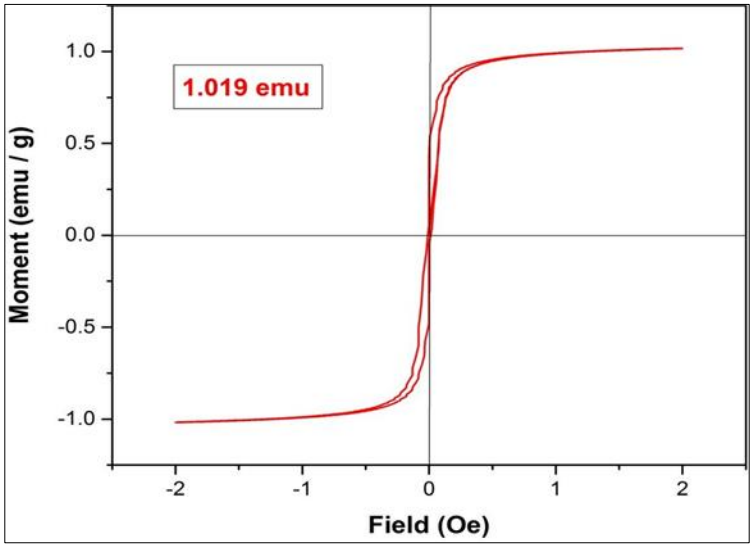

Figure 6 VSM Image of Iron Oxide Nanoparticles

All the haematological parameters of Mrigal are gradually increased from feed I to feed VI (Table 6). Total protein, carbohydrate and lipid content in Mrigal's muscle, gill and liver is higher in Feed VI (50 mg of iron oxide nanoparticles) when compared to other feeds. (Table 7).

Table 6 Haematological Parameters of Mrigal

Blood Parameters	Feed I	Feed II	Feed III	Feed IV	Feed V	FeedVI
RBC count (millions/cumm)	0.0	0.1	0.2	0.4	0.5	0.6
Haemoglobin (gm/dl)	0.1	0.3	0.6	0.9	1.4	1.8
Haematocrit(%)	0.0	1.0	2.0	3.0	4.0	5.0
MCV	97	130	160	170	179	189
MCH	36	54	66	67	70	72
MCHC	20	43	40	39	37	35
WBC count (Cells/ cumm)	3,000	2,000	2,500	3,200	4,300	5,500
Platelets count (Lakhs/cumm)	3,000	26,000	30,000	30,000	70,000	90,000

RBC - Red blood corpuscle WBC - White blood corpuscle; MCV - Mean corpuscular volume MCH - Mean corpuscular hemoglobin; MCHC - Mean corpuscular haemoglobin concentration.

Table 7 Biochemical parameters of Mrigal exposed to the different quantities of Iron Oxide nanoparticles

Quantity of Iron oxide Nanoparticles(mg)	Organs	Protein (mg/g)	Carbohydrate (mg/g)	Lipid (mg/g)
Feed I (Control)	Muscle	1.5	1.04	0.63
	Gill	1.02	0.57	0.51
	Liver	1.03	0.09	0.67
Feed II	Muscle	1.30	0.84	0.79
	Gill	2.49	0.46	0.64
	Liver	1.70	0.13	0.86
Feed III	Muscle	2.22	0.98	0.81
	Gill	4.53	0.55	0.55
	Liver	2.35	0.24	0.91
Feed IV	Muscle	2.74	1.33	0.90
	Gill	6.2	0.76	0.65
	Liver	2.88	0.26	1.33
Feed V	Muscle	3.37	1.56	1.15
	Gill	7.99	0.81	0.72
	Liver	2.89	0.35	1.13
Feed VI	Muscle	3.99	1.60	1.25
	Gill	8.49	1.04	0.99
	Liver	3.22	0.58	1.52

4. Discussion

Synthesized iron oxide nanoparticles' UV-Visible absorbance was at a peak ranging from "328 nm". The maximum absorbance of synthesized iron oxide nanoparticles peaks at "241 nm". A slight shift at "260 nm" confirmed the presence of phenol compounds and the formation of iron oxide nanoparticles may due to the presence of polyphenols complex present at "290 nm" [12].The SEM image shows that the obtained nanoparticles are hexagonal and cluster shape in

nature with agglomerated and iron oxide elements present in the synthesized iron oxide nanoparticles. The microscopic image shows that the Fe_3O_4 nanoparticles did not appear as discrete particles but from much larger dendritic flocks whose size could be reached a micron-scale size range of about 11.27 (scale bar 1 μm). Hasany *et al.,*(2012)[13] reported the morphology and size of nanoparticles using SEM. Iron oxide nanoparticles were more aggregates. This agglomeration can be avoided by the use of the calculations process to get a clear morphological structure and particle shape[14].In this study, the EDAX spectrum of iron oxide nanoparticles is shown as two peaks located between 0.2KeV and 6. 4KeV.The maximum peak located on the spectrum at 6.4KeV clearly comes from iron. The second peak located on the spectrum at 0.2 KeV clearly indicates oxygen. It confirms that the chemically synthesized iron oxide Nps has strong peaks of Fe and O. The EDAX spectrum iron oxide nanoparticles are shown as two peaks located between 2KeV and 10 Kev and maximum related to the iron characterized lines K[15].The XRD result was viewed the crystalline nature. The XRD diffraction peaks of FeO nanoparticles are indexed as 2702,2915,3223,3033,3065,2865. Suganya *et al.,* (2016)[16] reported that the synthesized iron oxide nanoparticles are crystal in nature and further confirmed by XRD and the nano-crystal average size is 10 to 16 nm. Chen *et al.* (2014)[17] reported that synthesized Fe_3O_4 Nps are crystalline in nature through XRD analysis. FTIR measurement was carried out to identify the functional groups and bands from 4000-400. Fourier Transform Infrared Spectroscopy results revealed the functional groups of iron oxide nanoparticles and viewed the functional groups of N-H Aliphatic primary amine, C-H Alkane, C-C Alkene, C-O Alkyl aryl ether, C=C Alkene, C-I Halo compound, C-I Halogen Compound stretching of proteins. Arokiyaraj *et al.,* (2013) [18] reported the main functional group of iron oxide nanoparticles contains alcohol, phenols and a primary amine. Similar results were obtained from the FT-IR spectrum of iron oxide nanoparticles conformation peak at 511-535 cm[19]. FT-IR spectra were recorded in the spectral range of 500-4500 cm of the as-received PAN powder, pure PAN fibres, and nanocomposite fibres with different particle loadings, respectively. Also reported that the FTIR spectrum of iron oxide nanoparticles was analyzed in a range from 4000-400cm[15]. A functional group such as N-H stretching, PO- symmetric stretch, nucleic acid COO symmetric stretch and amino acids, C-O asymmetric stretching of glycogen. The magnetic properties of iron oxide nanoparticles were measured by Vibrating Sample Magnetometer (VSM). In the present study, the saturation magnetization (Ms) of the Fe_2O_3 nanoparticle is 1.019emu/gm. Other researchers reported that the saturation magnetization (MS) is 1.019 emu/gm for iron oxide nanoparticles[20]. Ferromagnetic properties were already reported by many authors in different methods [21,22,23,24].

Condition factor was higher in Feed II when compared to other iron oxide nanoparticles supplemented feeds. A Similar condition factor was also reported in Common carp fed with iron oxide nanoparticles [25]. Feed consumption and feed conversion efficiency of Mrigal were higher in feed III and lower in feed VI. The Feed conversion ratio was good in Ex Feed III. Mukesh Mehta Ambani (2015) [26] reported that the feed conversion ratio was higher in control when compared to different concentrations of prepared feed of *Macrobrachium rosenbergii*. The Feed conversion ratio was good in EX. Feed II & III. The feed conversion ratio was higher in the control and lower in the zinc oxide fed of *Macrobrachium rosenbergii* [27]. Growth was higher in Feed V when compared to control and significantly increased the Mrigal growth. The specific growth rate was gradually increased in lower concentrations to higher concentrations of zinc-supplemented feed of *Penaeus vannamai* [28]. Assimilation and metabolism of Mrigal are higher in feed III. The Gross growth efficiency and Net growth efficiency of Mrigal are higher in feed I.

Haematological parameters are very helpful in the judgment of the health condition of fish species haematological parameters such as WBC, RBC, Hb, Hct, lymphocytes and eosinophils are gradually increased when the iron oxide nanoparticles increased. Also, a high concentration of selenium nanoparticles supplemented feed has increased the blood parameters when compared to the control group of African catfish, *Clarias gariepinus* [29]. The haematological parameters of *Labeo rohita* exposed to 1 and 25 mg/l of Fe3O2 Nps showed a significant ($P<0.05$) decrease [30].

Total protein content in muscle, liver and gill exposed to Mrigal significantly increased with an increased quantity of iron oxide nanoparticles. The total protein and lipid content in the muscle and gill of zebrafish gradually increased when the number of iron oxide nanoparticles increased[31]. The iron oxide nanoparticles altered the biochemical parameters of *Labeo rohita* [15]. Also reported that the selenium nanoparticles in the feed increased the protein, carbohydrate and lipid content of muscle, gill and liver on Crucian carp *Carassius auratus*[32].

5. Conclusion

The results conclude that feed III containing 20 mg of Iron oxide nanoparticles was suitable for the growth and feed VI containing 50 mg of Iron oxide nanoparticles enhanced the haematological and biochemical parameters of Mrigal.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflicts of interest regarding the publication of this paper.

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