RESEARCH ARTICLE Agricultural Science Digest, Volume 41 Issue 2: 338-344 (June 2021)

Evaluation of Different Quantity of Iron Oxide Nanoparticles on Growth, Haematological and Biochemical Characteristics of Koi Carp

K. Sangeetha, M.R. Rajan

10.18805/ag.D-5236

ABSTRACT

Background: Nanoparticles such as Se, Cu, Fe, FeO, Zn and ZnO play a vital role in aquaculture operations and are essential microminerals to enhance fish growth. The dietary supplementation of nanoparticles produces better survival, growth, antioxidant levels and immunity in aquatic organisms. The present work aimed to evaluate the different quantity of Iron oxide nanoparticles on the Growth, Haematological and Biochemical Characteristics of Koi carp.

Methods: Iron oxide nanoparticles were synthesized and characterized by using SEM, EDAX, FT-IR, XRD and VSM. Different quantity of iron oxide nanoparticles such as 0, 10,20,30,40 and 50mg/100g-1 was prepared along with the fish meal, groundnut oil cake, wheat flour and tapioca flour for feed I(Control), II, III, IV, V and VI respectively. Feed utilization, hematological and biochemical composition of Koi carp *Cyprinus carpio var.koi* were estimated after 21 days.

Result: The feed consumption, feed conversion efficiency, feed conversion ratio, Growth and Specific Growth Rate, Gross and Net Growth Efficiency of Koi carp was higher in Ex. Feed IV. The Hematological parameters of Koi carp gradually increased from Feed I to VI. Total protein, carbohydrate and lipid in muscle, gill and liver of Koi carp were higher in Feed VI and lower in feed I. The present work suggests that growth parameters are better in feed IV and hematological and biochemical parameters are higher in feed VI.

Key words: Biochemical, Growth, Hematological, Iron oxide nanoparticles, Koi carp.

INTRODUCTION

Aquaculture is the world's fastest-growing sector of agriculture. Presently, India ranks second in terms of total fish production with an annual fish production of about 9.06 million metric tonnes. Among aquaculture, ornamental fish culture is the culture of attractive colorful fishes. Among different ornamental fishes, Koi carp is one of the most popular, favorite ornamental fish and it has high market value because of its excellent color. The fisheries and aquaculture industry can be revolutionized by using nanotechnology tools for rapid disease detection, targeted delivery of drugs, DNA vaccines and nutrients. Nanoparticles such as Se, Cu, Fe, FeO, Zn and ZnO play a vital role in an aquaculture operation. The dietary supplementation of nanoparticles produced better survival, growth, hematology, biochemical, antioxidant levels and immunity in aquatic organisms. Iron is necessary by most of the living organisms because it is required for the metabolic process including oxygen transport, drug metabolism, steroid synthesis, DNA synthesis, ATP production, electron transport and cellular respiration (Crichton *et al*, 1991). The iron content of fish is very low compared to that of mammal. Although the gill membrane absorbs iron to a certain extent, the intestinal mucosa is considered to be the major site. The work related to the evaluation of different quantities of iron oxide nanoparticles on growth, hematological and biochemical characteristics of Koi carp is wanting. Hence the present work was carried out.

Department of Biology, The Gandhigram Rural Institute- Deemed to be University, Gandhigram-624 302, Tamil Nadu, India.

Corresponding Author: M.R. Rajan, Department of Biology, The Gandhigram Rural Institute- Deemed to be University, Gandhigram-624 302, Tamil Nadu, India. Email: mrrrajanbio@gmail.com

How to cite this article: Sangeetha, K. and Rajan, M.R. (2021). Evaluation of Different Quantity of Iron Oxide Nanoparticles on Growth, Haematological and Biochemical Characteristics of Koi Carp. Agricultural Science Digest. 41(2): 338-344. DOI: 10.18805/ag.D-5236.

Submitted: 23-06-2020 **Accepted:** 24-10-2020 **Online:** 19-01-2021

MATERIALS AND METHODS

Synthesis and characterization of iron oxide nanoparticles

The co-precipitation method was used for the synthesis of iron oxide nanoparticles. The aqueous solution of $FeCl_2$ and $FeCl_3$ was prepared in a 1:2 ratio. NaOH (0.1N) was added drop by drop under constant stirring for 30 minutes, the solution turns brownish yellow color. The yellow color of the aqueous solution turned into greenish-black precipitated by the addition of NaOH when the pH was 12. Then it was centrifuged at 5000 rpm for 3 minutes with ethanol and the collected iron oxide nanoparticles were allowed to air dry/ dissolve with distilled water for 8 hr sonication (50Hz frequency Vibronics -230V) (Fig 1). The morphology of the $Fe2O_3$NPs was determined by using a scanning electron microscope (SEM) (LEO 1455 VP). An energy-dispersive

X-ray detection instrument (EDAX) (HORIBA 8121-H) was used to examine the elemental composition of the sample. Structure and crystalline size of Fe_3O_4 nanoparticles were determined by XRD using an X-ray diffractometer with nickel –filter CuKα radiations in the 20 range (λ=1.5418Å) from an X-ray tube run at 40kV and 30ma. The FT-IR measurement was carried out to identify the functional groups of the bioactive components based on the peak value in the region of infrared radiation. The magnetic property of Fe_2O_3 nanoparticles was determined by a vibrating sample magnetometer (VSM).

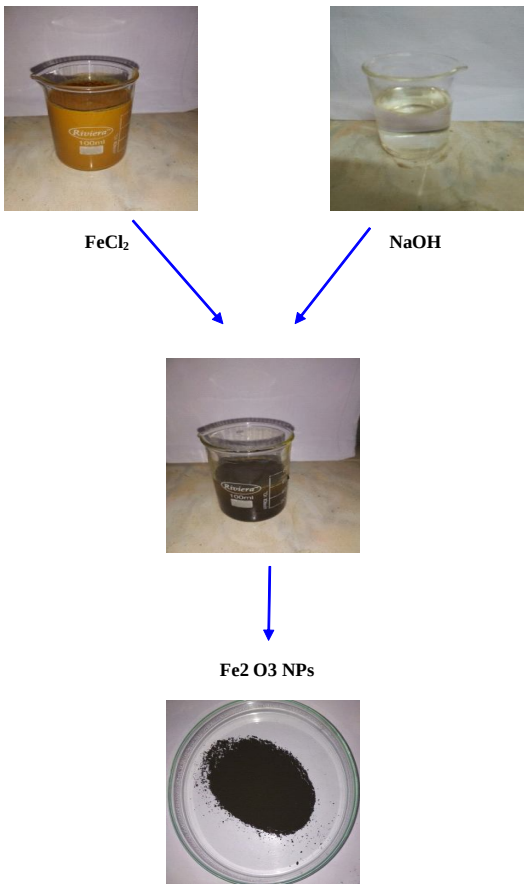

Fig 1: Synthesis of iron oxide nanoparticles.

Collection and acclimation of Koi carp

For growth work, Koi carp fish fingerlings (1.504 ± 0.30g) were collected from Aqua garden, Kadachanenthal, Madurai, Tamil Nadu, India and transported to the laboratory in polythene bags filled with oxygenated water. Fishes were acclimated in glass aquaria (60×45×45 cm) for 15 days at 28±2°C and fed 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

The raw materials are selected based on their ability to supply nutrients such as proteins, carbohydrates and fat at the low cast. Fish meal and Groundnut Oil Cake were used as protein sources; wheat flour and tapioca flour were used as carbohydrate sources; vegetable oil used as lipid source and served as binding agents and suppletive mix were used. After knowing the protein content by the Micro-Kjeldahl method (Jeyaraman, 1992) the raw materials were ground and sieved through a 425-micron sieve. The ingredients were weighed and mixed thoroughly with 130-150ml of distilled water. The mixed feedstuff was put in an autoclave for 15min at 100°C and cooled. After cooling, fish oil, sunflower oil, suppletive-mix, sodium chloride, sodium benzoate and different quantity of iron oxide nanoparticles such as 0, 10, 20, 30, 40 and 50mg/100g^{-1} were mixed for feed I, II, III, IV, V and VI and it was extruded with the help of pelletizer. The pellets were dried at room temperature. The formulated feed was kept in an airtight container at -20°C until used to prevent contamination (Table 1).

Design for growth studies

For the present work, the uniform size of Koi carp (*Cyprinus carpio var.koi*) (1.504±0.30 g) were selected and introduced in rectangular glass tanks (45cm×22cm×22cm) having a capacity of 18 liters. Five fishes were introduced in each tank and each treatment triplicates were maintained. During rearing, the fishes were fed on the 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 fishes and dried to constant

Table 1: Composition of different experimental feeds (g/100g).

Ingredients	Experimental feeds					
	Feed I (control)	Feed II	Feed III	Feed IV	Feed V	Feed VI
Fishmeal	36.2	36.2	36.2	36.2	36.2	36.2
Groundnut oil cake	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
Iron oxide nanoparticles	0	10mg	20mg	30mg	40mg	50mg

weight. The fecal matter was collected daily before changing the water with the least disturbance to the fishes and dried at 95°C. 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 fishes were measured in live conditions. Collected Muscle, Gill and Liver from all treatments for further analysis. Condition factor (k), survival rate and feed utilization parameters such as feed consumption, feed conversion efficiency, feed conversion ratio, specific growth rate, assimilation, metabolism, gross growth efficiency and net growth efficiency were estimated after 21 days.

Hematological assay

Blood samples were collected from the anus point on the right side of the fish. Complete blood parameters such as WBC, Hemoglobin, RBC, Hematocrit (PCV), MCV, MCH, MCHC and platelet count were estimated.

Biochemical characteristics of muscle, gill and liver of Koi carp

Total protein (Lowry *et al*., 1951), carbohydrate (Carrol *et al*., 1956) and lipid (Barnes and Blackstock, 1973) were estimated.

The whole work was carried out in the Department of Biology, The Gandhigram Rural Institute- Deemed to be University, Gandhigram, India between September 2018 and April 2019.

RESULTS AND DISCUSSION

Iron oxide nanoparticles were synthesized by co-precipitation of Fe2+ and Fe3+ by the addition of a base. The size, shape and composition of iron oxide nanoparticles synthesized through chemical methods depend on the type of salt used, Fe2+ and Fe3+ ratio, pH and ionic strength (Ali *et al*., 2016). Complete precipitation of Fe_3O_4 was expected between pH 9 and 14 (Gupta and Wells, 2004) and the precipitate was black (Cornell and Schertmann, 1991). Scanning Electron Microscopy indicates that nanoparticles formed as agglomerated because of the adhesive nature of distorted irregular cluster appearance (Fig 2). The diameter of iron oxide NPs is calculated in the range of 50-90 nm where the average diameter of iron oxide NPs is close to 60+10 nm. Poedji Loekitowati Harini *et al*. (2013) reported that the SEM image of synthesized iron oxide nanoparticles has a clear image of the cluster shape ranges from 30nm to 100nm. EDAX spectrum recorded on the iron oxide nanoparticles is shown as two peaks located between 0.5 KeV and 8 KeV (Fig 3). The maximum peak located on the spectrum at 6.4 KeV clearly shows iron. The second maximum peak located on the spectrum at 0.5 KeV indicates oxygen. The composition of iron and oxygen is 70.86% and 29.14%. Keerthika *et al*. (2017) reported that the EDAX spectrum iron oxide nanoparticles show three peaks located between 2KeV and 10KeV. The phase structure of synthesized iron oxide nanoparticles is shown in Fig 4.

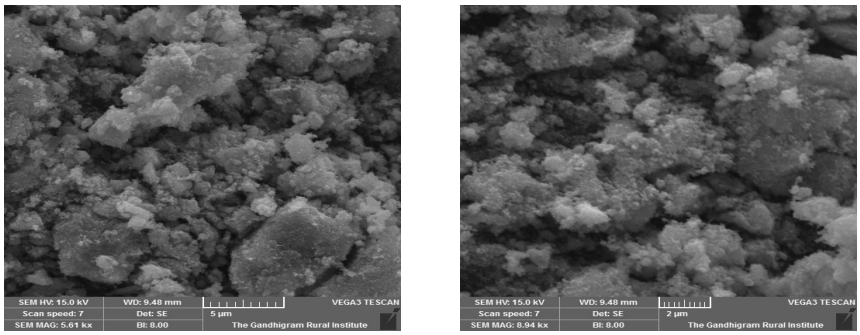

Fig 2: SEM image of Iron oxide nanoparticles.

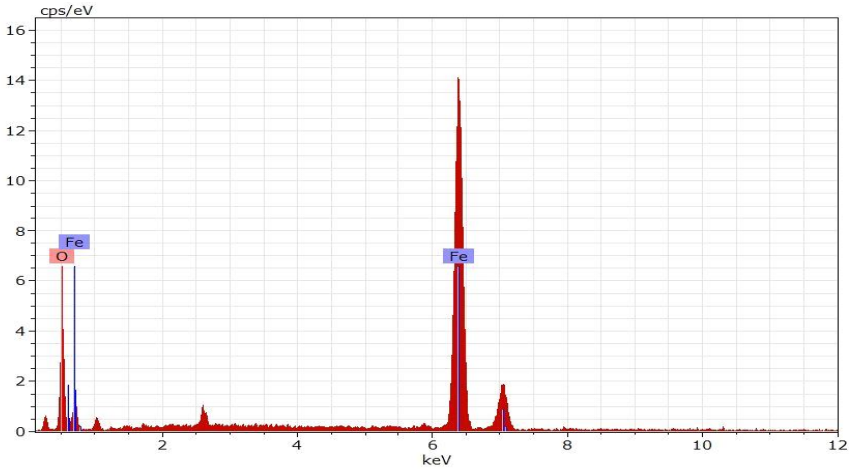

Fig 3: EDAX Image of iron oxide nanoparticles.

The 2θ value of 24.150, 33.150, 35.610, 40.790, 54.070 and 64.060 in the reference element iron (JCPDF No: 089-8104) which are attributed 012, 104, 110, 113, 024, 116, 214 and 300 crystallographic plane of face-centered cubic iron crystals. These results indicated that iron oxide nanoparticles are cubic and also supported by the XRD results which indicate that the γ-Fe_2O_3 phase contains only Fe3+ cations. Furthermore, the approximate crystallite size (D) of the iron oxide nanoparticles was calculated using the Scherer equation and approximate particle size 20+25 nm. Carmen *et al*. (2012) also reported γ-Fe_2O_3 and the particle size was calculated using the Scherer equation. FT-IR measurement was carried out to identify the functional groups of the bioactive components based on the peak value in the region of infrared radiation. Iron oxide formation was confirmed by 3456.24, 2928.47, 1628.25 and 555.13 cm^{-1} bands have -OH stretching, primary amines, alcohol, phenols and alkyl halides. (Fig 5). Arokiyaraj *et al*., (2013) reported that the main functional group of iron oxide nanoparticles are alcohol, phenols and primary amines. The magnetic properties of synthesized nanoparticles in the presence of a magnetic field were measured using a vibrating sample magnetometer. Fig 6 shows that the saturation magnetization (Ms) of Fe_2O_3 nanoparticle is 1.019 emu/gm. Ying Wang *et al*. (2013) also reported that the saturation magnetization (MS) is 1.019 emu/gm for iron oxide nanoparticles. Ferromagnetic properties were already reported by many authors in different methods (Pei *et al*., 2007, Lopez *et al*., 2012 and 2014 and Zhuang *et al*., 2014).

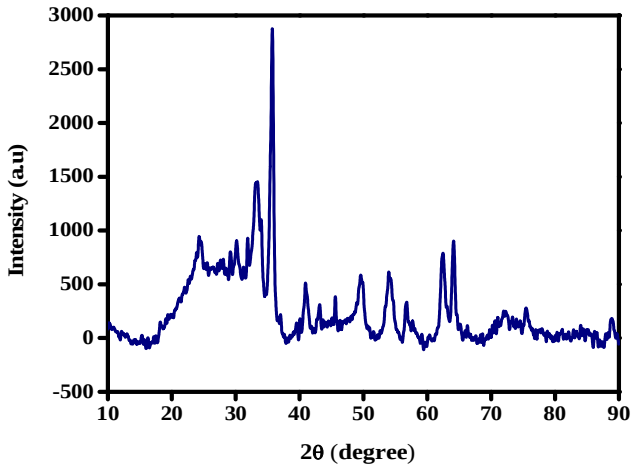

Fig 4: XRD Image of iron oxide nanoparticles.

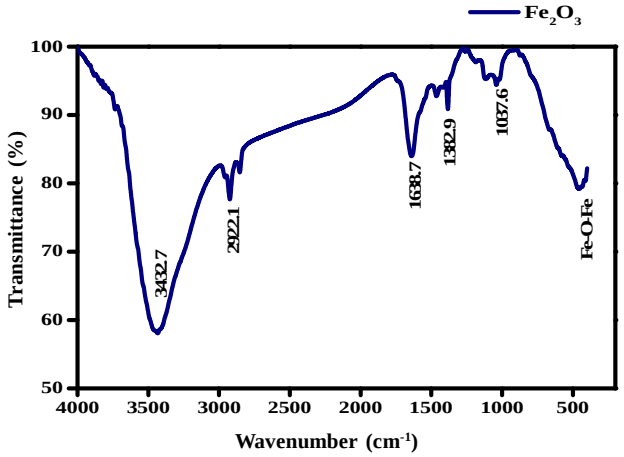

Fig 5: FT-IR Image of iron oxide nanoparticles.

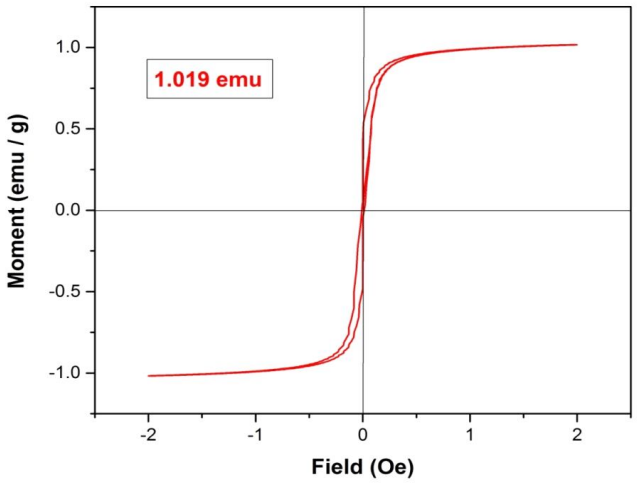

Fig 6: VSM Image of iron oxide nanoparticles.

The condition factor was higher in Feed III when compared to other iron oxide nanoparticles supplemented feeds (Table 2). Srinivasan *et al*., (2016) reported an increase in the condition factor of *Macrobrachium rosenbergii* post-larvae fed with 40g/ kg^{-1} of iron oxide nanoparticles in the feed. Survival, feed utilization and growth parameters of Koi carp about the different quantity of Iron oxide nanoparticles are presented in Table 3. Survival rate of Koi carp was 80 to 90% in 20, 30 and 50mg of iron oxide supplemented feeds. A similar survival rate was also reported in common carp fed with iron oxide nanoparticles (Anand Sadanandan Ramya *et al*., 2014). Feed consumption and feed conversion efficiency of Koi carp were higher in feed VI (3.26 + 0.57and 0.28 +0.08) containing 50 mg /g^{-1} of iron oxide nanoparticles and lower in feed I (control). Amina Zuberi *et al*. (2015) reported that the feed consumption and feed conversion in Juvenile Grass Carp (*Ctenopharyngodon idella*) increased with an increase in the concentration of Zinc nanoparticles. Hayat *et al*. (2007) also reported the increase of growth parameters except feed conversion ratio in major carps *viz*. *Catla catla, Labeo rohita* and *Cirrhinus mrigala*. The feed conversion ratio was good in Ex. Feed IV (3.15) containing 30 mg of iron oxide nanoparticles. Mukesh Mehta Ambani

Table 2: Condition factor (k) of Koi carp.

Feeds	Initial	Final
EX. Feed I (control)	3.90±0.24	4.06±0.25
EX. Feed II (10mg)	4.21±0.22	4.58±0.22
EX. Feed III (20mg)	4.6±0.29	4.96±0.35
EX. Feed IV (30mg)	4.08±0.7	4.48±0.26
EX. Feed V (40mg)	3.25±0.21	4.01±0.35
EX. Feed IV (50mg)	4.45±0.48	4.84±0.75

(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*., (2016) reported that the feed conversion ratio was higher in control and lower zinc oxide fed with Macrobrachium rosenbergii. Growth and Specific Growth rate were higher in Feed IV (30 mg/g-1) when compared to control and significantly increased the Koi carp growth. Davis *et al*., (1993) and Gammanpila *et al*., (2007) also reported that the specific growth rate was gradually increased in lower concentration to a higher concentration of zinc supplemented feed of *Penaeus vannamai*. The assimilation of Koi carp is higher in feed IV and metabolism is higher in feed II. Gross growth efficiency of Koi carp is higher in feed IV and net growth efficiency of Koi carp is higher in feed V. Feed consumption, growth, gross and net growth efficiency of Koi carp is significantly varied (Table 4).

Hematological parameters are very helpful in the judgment of the health condition of fish species. The complete blood count of Koi carp progressively increased when the quantity of iron oxide nanoparticles increased (Table 5). Anand Sadanandan Ramya *et al*., (2015) reported

Table 3: Survival, feed utilization and growth parameters of Koi carp in relation to different quantity of Iron oxide nanoparticles.

Parameters	Experimental feed					
	Feed I (control)	Feed II (10mg)	Feed III (20mg)	Feed IV (30mg)	Feed V (40 mg)	Feed VI (50mg)
Survival rate (%)	100	100	80±1.02	90±1.12	100	90±1.15
Feed consumption (FC)(g/g live wt/21 days)	2.36±0.26[a]	2.46±0.36[b]	2.83±0.89[c]	2.96±0.46[d]	3.1±0.92[e]	3.26±0.57[f]
Feed conversion efficiency (FCE)	0.09±0.03	0.12±0.01	0.15±0.05	0.21±0.06	0.25±0.02	0.28±0.08
Feed conservation ratio (FCR)	4.82±0.61	5.26±0.23	6.52±0.52	3.15±0.35	4.93±0.56	6.33±0.72
Growth (G)gm/gm live wt/21days	0.35±0.05	0.46±0.08	0.53±0.05	0.85±0.06	0.67±0.08	0.74±0.05
Specific growth rate (SGR)(%)	1.45±0.15	2.05±0.13	0.96±0.19	3.9±0.25	2.98±0.52	1.82±0.63
Assimilation (A)	1.44±0.32	0.89±0.55	1.28±0.66	1.66±0.47	0.93±0.9	1.12±0.49
Metabolism (M)	1.86±0.25	2.1±0.63	1.9±0.32	1.8±0.4	1.7±0.57	1.86±0.24
Gross growth efficiency (GGE)(%)	39.8±3.96[a]	44.4±4.19[b]	48.7±4.72[c]	49.2±2.81d	41.8±4.6[e]	36.6±2.49[f]
Net growth efficiency (NGE)(%)	30.03±4.16	26.1±1.79	33.9±1.04	34.96±2.6	35.02±1.69	30.07±2.21

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

Each value is the average (±SD) performance of 5 individuals in triplicates reared for 21 days.

Table 4: ANOVA (Analysis of Variance) of growth parameters (Feed consumption, growth, gross growth efficiency, net growth efficiency) of Koi Carp.

Parameters	Source	SS	Df	MS	F	PROB
Feed consumption	Columns	1.19256	05	0.34321	5.78	0.0231
		0.70056				
	Errors	1.56743	12	0.06008		
	Total	3.46055	18			S
Growth	Columns	0.89065	05	0.20314	9.78	0.0008
		0.2453				
	Errors	0.98765	12	0.02237		
	Total	2.1236	18			S
Gross growth efficiency	Columns	1989.78	05	478.78	7.65	0.0019
		768.87				
	Errors	2654.87	12	52.765		
	Total	5413.52	18			S
Net growth efficiency	Columns	1852.44	05	345.98	9.78	0.0007
		364.82				
	Errors	1328.8	12	35.897		
	Total	3546.06	18			S

Table 5: Hematological parameters of Koi carp.

Blood parameters	Feed I	Feed II	Feed III	Feed IV	Feed V	Feed VI
WBC (CELLS/CUMM)	54,000	17,600	37,500	51,700	68,700	71,000
Hemoglobin(gm/Di)	0.4	0.8	1.8	2.1	2.6	2.9
RBC (Count)(Millions/cmm)	0.1	0.1	0.2	0.3	0.4	0.5
Hematocrit (PCV)(%)	1.3	2.2	4.4	5.2	5.8	6.7
MCV	98	132	165	169	171	187
MCH	38	56	67	68	69	70
MCHC	21	42	41	40	38	39
Platelets count (Cells/Cumm)	30,000	50,000	68,000	73,000	86,000	94,000

Table 6: Total Protein, carbohydrate and lipid of Koi carp.

Feed		Protein (mg/g)	Carbohydrate (mg/g)	Lipid (mg/g)
I	Muscle	1.32	1.5	1.23
	Gill	0.98	1.03	1.04
	Liver	1.02	0.08	0.66
II	Muscle	1.87	2.6	1.69
	Gill	1.56	1.56	0.88
	Liver	1.69	0.12	0.95
III	Muscle	2.10	2.2	1.89
	Gill	3.45	1.04	1.56
	Liver	2.34	0.23	0.50
IV	Muscle	2.22	2.0	2.02
	Gill	3.33	0.56	1.67
	Liver	2.87	0.28	1.32
V	Muscle	3.12	1.9	2.56
	Gill	4.60	0.83	1.77
	Liver	2.88	0.14	1.12
VI	Muscle	3.35	2.8	2.87
	Gill	5.02	1.9	1.80
	Liver	3.21	0.67	1.51

that the hematological parameters were gradually increased with different doses of iron oxide nanoparticles fed on Indian major carp. Abdel *et al*. (2007) also reported the increase of blood parameters when compared to control with a high concentration of selenium nanoparticles supplemented feed to African catfish, *Clarias gariepinus*. Saravanan *et al*. (2015) reported that the hematological parameters of *Labeo rohita* exposed to 1 and 25 mg/l of Fe_3O_2 Nps showed a significant ($P<0.05$) decrease.

Total protein, carbohydrate and lipid of muscle, gill and liver of Koi carp gradually increased when the concentration of iron oxide nanoparticles increased (Table 6). Keerthika *et al*. (2017) reported that the iron oxide nanoparticles altered the biochemical parameters of *Labeo rohita*. Muralisankar *et al*. (2016) reported the concentration based increase and decrease of protein, lipid and carbohydrate contents in Zn supplemented diet fed to *Macrobrachium rosenbergii*. Ashouri *et al*. (2015) also reported that the selenium nanoparticles in the feed have increased the protein, carbohydrate and lipid content of muscle, gill and liver of Crucian carp (*Carassius auratus*).

CONCLUSION

The findings strongly suggest that feed IV containing 30mg Iron oxide nanoparticles was sufficient for the growth of Koi carp and feed VI containing 50 mg of Iron oxide nanoparticles are suitable for the enhancement of hematological and biochemical parameters.

Conflict of interest

The authors declare no conflict of interest.

ACKNOWLEDGEMENT

Authors thank the Department of Biology, The Gandhigram Rural Institute-Deemed to be University, Gandhigram, Tamil Nadu, India for offering laboratory facilities to carry out this work.

REFERENCES

Abdel, M., Abbass, F. and Mousa, M. (2007). Growth performance and physiological response of African Catfish, *Clarias gariepinus* (B) fed organic selenium prior to the exposure to environmental copper toxicity. Aquaculture. 272(1-4): 335-345.

Ali, A., Zafar, H., Zia, M., Haq, I.U., Phull, A.R., Ali, J.S., Hussain, A. (2016). Synthesis, characterization, applications and challenges of iron oxide nanoparticles. Nanotechnology, Science and Applications. 9: 49-67.

Amina Zuberi., Faiz. Muhammad Rauf., Samina Nazir. (2015). Zinc oxide, Zinc sulfate and Zinc oxide nanoparticles as a source of dietary zinc: Comparative Effects on growth and hematological indices of juvenile Grass Carp (*Ctenopharyngodon idella*). International Journal of Agriculture and Biology. 17(3): 568-574.

Anand Sadanandan Ramya, Mathan Ramesh., Manoharan Saravanan, Rama Krishnan Poopal, Subramanian Bharathi, Devaraj Nataraj (2014). Effect of Iron oxide nanoparticles on certain hematological, biomedical, food additives, iron Regulatory and gill Na+/k+ ATPase activity of an Indian major carp. Journal of King Saud University-Science. 27: 151-160.

Arokiyaraj, S., Saravanan, M., Udaya Prakash, N.K., Valan Arasu, M., Vijayakumar, B., Vincent, S. (2013). Enhanced antibacterial activity of iron oxide magnetic nanoparticles treated with *Argemone mexicana* L. leaf extract: An *in vitro* study Materials. Research Bulletin. 48: 3323-3327.

Ashouri, S., Keyvanshokooh, S., Salati, A.P Johari, S.A., Zanoosi, H.A. (2015). Effects of Different levels of dietary selenium nanoparticles on growth performance, muscle Composition, blood biochemical profiles and antioxidant status of common carp (*Cyprinus carpio*). Aquaculture. 446(1): 25-29.

Carmen., Coralia Bleotu., Simona liliana. (2012). Magnetic properties and biological activity evaluation of iron oxide nanoparticles. Journals of Nanomaterials. 893970.

Carrol, N.V., Longley, R.W., Roe, J.H. (1956). The determination of Glycogen in Liver and Muscle by use of Anthrone Reagent. Journals of Biological Chemistry. 200: 583-593.

Crichton, Dominic, Florence, Roberta. (1991).Chemical and structural characterization of iron cores of haemosiderins isolated from different sources. European Journal of Biochemistry. 209(3): 847-850.

Davis, D.A., Lawrence, A.L., Galtin, D.M. (1993).Evaluation of the dietary zinc requirement of *Penaeus vannamei* and effects of phytic acid on zinc and phosphorus bioavailability. Journal of World Aquacultural Society. 24: 40-47.

Gammanpila, M., Age, A.Y., Bart, A.N. (2007). Evaluation of the effects of dietary vitamin C, E and Zinc supplementation on reproductive performance of Nile tilapia (*Oreochromis niloticus*). Sri Lanka Journal of Aquatic Science. 12: 39-60.

Gupta, A.K. and Wells, S. (2004). Surface modified superparamagnetic nanoparticles for drug delivery: Preparation, characterization and cytotoxicity studies. Institute of Electrical and Electronics Engineers Transactions on Nanobioscience. 3(1): 66-73.

Hayat, S., Javed, M., Razzaq, S. (2007). Growth performance of metal stressed major carps *viz*. *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* reared under a semi-intensive culture system. Pakistan Veterinary Journal. 27: 8-12.

Jayaraman, J. (1992). Laboratory Manual in Biochemistry. Wiley Eastern Ltd, New Delhi. Fourth Reprint, pp. 75-78.

Keerthika, V., Ramesh, R., Rajan, M.R. (2017). Toxicity assessment of iron oxide nanoparticles in *Labeo rohita*. International Journal of Fisheries and Aquatic Studies. 5(4): 01-06.

Lopez, J., Espinoza-Beltran, F.J., Zambrano, G., Gomez M.E., Prieto, P. (2012). Characterization of magnetic nanoparticles of $CoFe_2O_4$ and $CoZnFe_2O_4$ prepared by the chemical co-precipitation method. Revista Mexicana de Fisica. 58 (4): 293-300.

Lopez, J., Gonzalez, L.E., Quinonez, M.F., Gomez, M.E., Porras-Montenegro, N., Zambrano, G. (2014). Magnetic field role on the structure and optical response of photonic crystals based on ferrofluids containing $Co0.25Zn0.75Fe_2O_4$ nanoparticles. Journal of Applied Physics. 115(19): 1-8.

Lowry, H., Niora. J., Rosebrough. A., Lowis Farr., Rose. J. (1951). Protein Measurement with the Folin Phenol Reagent. Journal Biology and Chemistry. 193: 265-275.

Mukesh Meta Ambani. (2015). Effects of diet substitution on growth performance, energy consumption and digestive enzymes in Macrobrachium rosenbergii post larvae. Advances in Aquaculture and Fisheries Management. 3(5): 241-248.

Muralisankar, T., Saravana Bhavan, P., Radhakrishnan, S., Seenivasan, C., Manickam, N., Srinivasan. V. (2016). Effects of dietary zinc on the growth, digestive enzymes activities, muscle biochemical compositions and antioxidant status of the giant freshwater prawn Macrobrachium rosenbergii. Aquaculture. 448: 98-104.

Pei, W., Kumada, H., Natusme, T., Saito, H., Ishio, S. (2007). Study on magnetite nanoparticles synthesized by chemical method. Journal of Magnetism and Magnetic Materials. 310(2): 2375-2377.

Poedji Loekitowati Hariani., Muhammad Faizal., Ridwan., Marsi., Dedi Setiabudidaya. (2013). Synthesis and Properties of Fe3O4 Nanoparticles by Co-precipitation Method to Removal of Procion Dye. International Journal of Environmental Science and Development. 4(3): 366-340.

Saravanan, M., Suganya, R., Ramesh. M., Poopal, R.K., Gopalan, N., Ponpandian. (2015). Iron oxide nanoparticles induced alterations in hematological, biochemical and iron lonoregulatory responses of an Indian Major Carp *Labeo rohita*. Journal of Nanopart Research. 17(6): 274.

Srinivasan, V., Saravana Bhavan, P., Rajkumar, G., Satgurunathan, T., Muralisankar, T. (2016). Effects of dietary iron oxide nanoparticles on the growth performance, biochemical constituents and physiological stress responses of the giant freshwater prawn *Macrobrachium rosenbergii* post larvae. International Journal of Fisheries and Aquatic Studies. 4(2): 170-182.

Ying Wang, Parvin Kaur, Augustine Tuck Lee Tan (2013). Iron oxide magnetic nanoparticles synthesis by atmospheric microplasmas. Plasma and Applications. 32: 1460343.

Zhuang, L., Zhang, W., Zhao, Y., Shen, H., Lin, H., Liang, J. (2014). Preparation and characterization of Fe_3O_4 particles with novel nanosheets morphology and magnetochromatic property by a modified solvothermal method. Scientific Reports. 5: 1-6.