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# Evaluation of dietary metallic iron nanoparticles as feed additive for growth and physiology of Bagridae catfish *Clarias batrachus* (Linnaeus, 1758)

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**Abstract**
Nano-particles have enormous potential in improving growth and immune functions of fish in aquaculture. The present investigation was carried out for a period of 60 days to evaluate metallic iron nanoparticles (Fe-NPs) as a fish feed additive in growth and physiological stimulation of Bagridae catfish *Clarias batrachus*. Fe-NPs were prepared by aquostic method and mixed with the fish feed at different concentrations in addition to the control. The 40 mg/kg feed of Fe-NPs gave the highest growth and feed utilization performance. Significantly higher total protein and lipid content of fish muscle were also found at 40 mg/kg feed of Fe-NPs. Serum total protein, cholesterol and triglyceride were found to increase in a dose dependent-manner and indicates better health condition for the fish group fed 40 mg/kg feed of Fe-NPs supplemented diet. However, stress indicators (Alanine aminotransferase, ALT; aspartate aminotransferase, AST; and alkaline phosphatase, ALP) were found to increase with increasing the Fe-NPs concentration compared to control group. The accumulation of Fe-NPs in muscle, liver and serum were increased in all the fish groups with increasing the concentration of Fe-NPs in diets and higher accumulation was found in serum of fish. Therefore, considering the fish growth, proximate composition and health condition, it can be concluded that 40 mg/kg feed of Fe-NPs is sufficient to ensure the growth and health of *C. batrachus*.

**Keywords:** Metallic iron nanoparticles, feed additives, growth and physiology, *Clarias batrachus*

## Introduction
Fish feed is an essential component of the inputs in fish culture. The feed used in fish culture should ensure growth, immunity and sound health of fish to achieve maximum benefit from the culture system. The quality of feed has been seen as a problem that is generally expressed at the farm level as poor yield performance and higher cost of production. Therefore, researchers were trying to improve the quality of feed by incorporating different growth promoters such as antibiotics as feed additives. However, after the ban of antibiotic as growth promoters in fish feed new strategies in feeding and health management in fish aquaculture practice have received much attention [1]. Now-a-days, feed additives in nano forms have been reviewed to have different effects from enhancing growth and immunity through antioxidant effect to their use in fewer amounts than its bulk counterparts which enhances ration criteria [2, 3]. Metallic nanoparticles (NPs) which include particles made from Au, Ag, Pt, Fe, and Cu are widely used due to their unique properties such as diverse surface chemistries and can be prepared into a variety of shapes and sizes [4].
Metallic iron nanoparticles (Fe-NPs) are of great interest due to their unique physicochemical properties and have a great potential in fish feed additives for growth [5]. Iron is an essential micronutrient involved in oxygen transport and cellular respiration through its oxidation-reduction activity and electron transfer [6]. Dietary iron supplementation is necessary and Roeder and Roeder [7] demonstrated that the growth of fish was affected by dietary iron level. Iron deficiency causes hypochromic microcytic anemia in brook trout, *Salvelinus fontinalis* [8] and common carp, *Cyprinus carpio* [9]. In addition, excessive iron levels can also be toxic and it includes reduced growth, increased mortality, diarrhea, and histopathological damage to liver cells [6]. Therefore, determining the optimal Fe-NPs feed concentration for growth and physiology of fish is a necessary task.

In the present study, in addition to quantifying dietary Fe-NPs requirement of the Bagridae catfish *Clarias batrachus*, we evaluated dose-dependent effects of Fe-NPs supplementation on growth performance, feed utilization, muscle biochemical composition, blood parameters and serum enzyme activity of fish at the end of the feeding trial.

## Materials and Methods

### Preparation of Nanoparticles

Preparation of iron nanoparticles were done in the laboratory of Department of Agronomy and Agricultural Extension, University of Rajshahi, Bangladesh. In the present study, the aquostic method was used to prepare the metallic iron nanoparticles [10]. A mixture of reagent (salt) and polymer surfactant in ethylene glycol (EG) and/ $H_2O$ was heated in an oil bath for several minutes and desired shapes and sizes of nanostructures in high yields were prepared by changing various experimental parameters such as concentrations of reagent surfactants (e.g. PVP), reluctant and solvents (EG or other polyols), gas bubbling, temperatures and heating rate [10]. The synthesized nanoparticles were purified by precipitation method. Crystal structures and growth mechanisms of nanoparticles were characterized by Atomic Force Microscopy (AFM) (Park system.XE-70, South Korea). Produce solutions were centrifuged at 6,000 rpm three times for 30 min to ensure complete collection of products catch time. The precipitates were collected and then re-dispersed in ethanol. Samples for AFM measurements were prepared by dropping a droplet of the colloidal solution on the glass slides.

### Formulation of Diet

Ingredients and proximate composition of prepared diet are shown in Table 1. All feed ingredients were purchased from local market and in laboratory they were grinded to acquire fine powder. The powdered and sieved feed ingredients were weighed out and mixed thoroughly in 6 different ratios for preparing six different diets 1 control and 5 different diets containing Fe-NPs at various doses such as 0 (control), 10 (Fe-$NPs_{10}$), 20 (Fe-$NPs_{20}$), 30 (Fe-$NPs_{30}$), 40 (Fe-$NPs_{40}$) and 50 (Fe-$NPs_{50}$) mg/kg dry feed weight. Then distilled water was added and blending well (5 min) until the mixture achieves a dough consistency. The dough was pelletized in a manual pelletizer fixed with 3 mm diameter and the pellets were collected in aluminum trays. A thermostatic hot air oven (Microsil INDIA, Universal Lab Product Co., Chennai, India) was used to dry the diets until the moisture content was reduced below 10%. After drying diets were kept at 20ºC until used.

**Table 1:** Ingredients and proximate composition of experimental diet

| Ingredients | g/kg | Proximate composition | (%)† |
|---|---|---|---|
| Fish meal[a] | 275 | Protein | 33.11±0.14 |
| Mustard oil cake[a] | 200 | Lipid | 9.38±0.03 |
| Soybean meal[a] | 125 | Carbohydrate | 36.45±0.44 |
| Maize bran[a] | 125 | Moisture | 7.07±0.01 |
| Wheat bran[a] | 90 | Ash | 11.23±0.60 |
| Rice bran[a] | 90 | | |
| Soybean oil[a] | 60 | | |
| Choline chlorid[a] | 2.5 | | |
| Fe-free premix[*b] | 32.5 | | |

†Values are presented as mean ± SD, n= 3

*Fe-free premix (mg/kg of premix): vitamin A-156000 IU, vitamin $D_3$-31200 IU, vitamin E-299, vitamin $K_3$-26, vitamin $B_1$-32.5, vitamin $B_2$-65, vitamin $B_6$-520, vitamin $B_{12}$-0.16, Nicotinic Acid-520, Folic Acid-10.4, Copper-130, Iodine-5.2, Manganese-780, Zinc-650 and Selenium-1.95.

[a] Ingredients parched from local market of Rajshahi, Bangladesh.

[b] Supplied by Reneta Animal Health Pharma Co. Ltd. Bangladesh.

### Collection and maintenance of fish

Juveniles of *C. batrachus* having an average weight of 5.23±0.07 g were purchased from Fish Seed Hatchery, Rajshahi and transported live in aerated plastic bags to the laboratory of Department of Fisheries, University of Rajshahi. Fishes were kept in a cemented tank having flow through system and were acclimatized for two weeks. During the acclimatization water quality parameters maintained in the optimum range temperature, 27-30ºC, with a photoperiod of 12-h light and 12-h darkness.

### Experimental Design

After an acclimatization period, healthy and uniform sized fishes were selected, individually weighed by using electronic top-loading balance and evenly distributed in eighteen fiber glass aquaria at 10 fish per aquarium with similar initial weight. The experiment was conducted as a Completely Randomized Design (CRD) with six treatments as control, Fe-$NPs_{10}$, Fe-$NPs_{20}$, Fe-$NPs_{30}$, Fe-$NPs_{40}$ and Fe-$NPs_{50}$ each with three replications. Fishes were fed daily (twice a day) with a feeding rate of 3% body weight. After feeding period, the diet remaining in each tank was collected by siphoning before the second day's feeding. A routine work of exchanging 50% water from each aquarium was done daily.

### Water quality analysis

Water temperature was measured using a Celsius thermometer. Water pH was measured using an electronic pH meter (Jenwary, 3020). Dissolved oxygen (DO) and ammonia were measured by using a portable aquaculture kit (Model FF2, HACH, USA).

### Growth and feed utilization parameters

All fish in different experimental groups were weighed at the end of 30 days feeding trial for estimation of growth. Growth parameters were calculated according to the following formulae:

Weight gain (g) = Final weight (g) – Initial weight (g)

Percent weight gain (%) =

$$\frac{\text{Final weight (g)} - \text{Initial weight (g)}}{\text{Initial weight (g)}} \times 100$$

Specific growth rate (%/day)

$$= \frac{ln\ \text{Final weight (g)} - ln\ \text{Initial weight (g)}}{\text{Study period}} \times 100$$

$$\text{Condition factor (CF)} = \frac{\text{Final weight (g)}}{\text{Final length (cm)}^3}$$

$$\text{Survival rate (\%)} = \frac{\text{Final fish number}}{\text{Initial fish number}} \times 100$$

$$\text{Food conversion ratio (FCR)} = \frac{\text{Feed given (dry weight)}}{\text{Total wet weight gain}}$$

$$\text{Food conversion efficiency (FCE)} = \frac{\text{Total wet weight gain}}{\text{Feed given (dry weight)}}$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Total weight gain (g)}}{\text{Protein intake (g)}}$$

$$\text{Protein productive value (PPV \%)} = \frac{PT - PI}{\text{Protein intake (g)}} \times 100$$

Where, PT = Protein content in fish carcass at the end, PI = Protein content in fish carcass at the start.

**Muscle Composition**

At the end of the study period, two fishes were randomly collected from each aquarium and were used for determination of proximate composition in laboratory of Department of Applied Chemistry, University of Rajshahi, Bangladesh. Fish tissue samples were dried and one gram of dried tissue samples was digested separately with 10 ml of $HNO_3$ in a microwave device (CEM MDS 2100). Following digestion, the samples was diluted with distilled water to make up 20 ml and filtered. Crude protein was estimated by micro- kjeldahl method [11]; crude lipid by petroleum ether extraction using the soxhlet method [12]; moisture and ash content were analyzed following the method of AOAC [13]. Muscle and liver iron content were determined by Flame Atomic Absorption Spectrometer (Shimadzu, AA-6800) in central lab of University of Rajshahi, Rajshahi, Bangladesh followed by digestion in Bangladesh Council of Scientific and Industrial Research (BCSIR), Rajshahi, Bangladesh.

**Serum biochemical profile**

At the end of feeding trial, two fish from each treated group was randomly selected for measurement of serum biochemical profile. The blood was drawn from caudal vein of individual fish and were transferred into sterile tubes without any addition of anticoagulant and kept for 3 hours in slanting position. Samples were centrifuged at 5000 rpm for 10 minutes at 4ºC. Sera were collected by one ml auto-pipette. The collected sera samples were stored in deep freeze at -20°C for serum biochemical studies. Biochemical parameters viz., total protein, cholesterol, triglyceride, high density lipoprotein (HDL), low density lipoprotein (LDL), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were estimated by atomic absorption spectrophotometry using the kits prepared by Crest Biosystems®. Serum iron content was estimated by Biuret and bromocresol green (BCG) dye binding method [14].

**Statistical Analysis**

The data were analyzed by one-way analysis of variance (ANOVA) using SPSS (20.0), followed by Duncan's multiple range test to compare the differences among treatments where significant differences ($P < 0.05$) were observed. Data were expressed as mean ± SD.

**Results**

Water quality in all aquariums were observed to be normal and remained within ranges allowing for high growth rate and production of *C. batrachus* (Table 2). Water temperature was ranged from 27.73±0.33 (Fe-$NPs_{40}$) to 28.03±0.57°C (Fe-$NPs_{20}$), dissolved oxygen from 5.87±0.26 (Control) to 6.07±0.14 mg/l (Fe-$NPs_{20}$), pH from 6.97±0.03 (Fe-$NPs_{30}$) to 7.16±0.04 (Fe-$NPs_{20}$) and ammonia ranged from 0.065 mg/l to 0.082 mg/ l and DOES ranged from 6.05 to 6.62 mg/l.

**Table 2:** Water quality parameters of experimental aquariums

| Treatments | Temperature ($^0$C) | DO (mg/l) | pH | Ammonia (mg/l) |
|---|---|---|---|---|
| Control | 27.93±0.53$^a$ | 5.87±0.26$^a$ | 6.99±0.11$^a$ | 0.001±0.000$^a$ |
| Fe-$NPs_{10}$ | 27.90±0.35$^a$ | 5.97±0.12$^a$ | 7.06±0.13$^a$ | 0.002±0.001$^a$ |
| Fe-$NPs_{20}$ | 28.03±0.57$^a$ | 6.07±0.14$^a$ | 7.16±0.04$^a$ | 0.002±0.001$^a$ |
| Fe-$NPs_{30}$ | 27.76±0.68$^a$ | 5.90±0.25$^a$ | 6.97±0.03$^a$ | 0.002±0.001$^a$ |
| Fe-$NPs_{40}$ | 27.73±0.33$^a$ | 6.04±0.13$^a$ | 7.09±0.15$^a$ | 0.001±0.001$^a$ |
| Fe-$NPs_{50}$ | 27.80±0.29$^a$ | 6.00±0.14$^a$ | 7.09±0.09$^a$ | 0.001±0.001$^a$ |

Values in the same row having similar superscript letter are not significantly different (P > 0.05).

The growth performance data of *C. batrachus* fed the diets containing various concentrations of iron oxide nanoparticles (Fe-NPs) for 8 weeks are presented in Table 3. Survival of each group was 100% and there was no significant difference among treatments ($P > 0.05$). Initial weight was also not significantly different at the beginning of the experiment. Final weight, Weight gain, %weight gain, specific growth rate and condition factor were significantly affected by dietary Fe-NPs concentrations in feed compared to control group ($P < 0.05$). All the growth parameters were found to increase as the dietary Fe-NPs levels increased up to 40 mg/kg feed of Fe-NPs. However, further increase in concentration of Fe-NPs in diets significantly reduced the growth performance compared to Fe-$NPs_{40}$ group (Table 3).

**Table 3:** Growth performance of *Clarias batrachus* fed Fe-NPs enriched diets

| Treatments | IW (g) | FW (g) | WG (g) | %WG | SGR (%/day) | CF | Survival (%) |
|---|---|---|---|---|---|---|---|
| Control | 5.18±0.06$^a$ | 6.55±0.33$^d$ | 1.36±0.38$^d$ | 26.35±7.62$^d$ | 0.17±0.04$^d$ | 1.56±0.21$^b$ | 100 |
| Fe-$NPs_{10}$ | 5.24±0.06$^a$ | 7.51±0.45$^c$ | 2.27±0.49$^c$ | 43.34±9.54$^c$ | 0.26±0.05$^c$ | 1.87±0.03$^{ab}$ | 100 |
| Fe-$NPs_{20}$ | 5.27±0.08$^a$ | 8.88±0.24$^b$ | 3.61±0.24$^b$ | 68.52±4.78$^b$ | 0.37±0.02$^b$ | 2.14±0.35$^{ab}$ | 100 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Fe-NPs$_{30}$ | 5.21±0.07$^{a}$ | 9.16±0.24$^{b}$ | 3.95±0.16$^{b}$ | 75.75±2.13$^{b}$ | 0.41±0.01$^{b}$ | 2.08±0.37$^{ab}$ | 100 |
| Fe-NPs$_{40}$ | 5.21±0.10$^{a}$ | 10.89±0.29$^{a}$ | 5.69±0.20$^{a}$ | 109.20±2.27$^{a}$ | 0.54±0.01$^{a}$ | 2.42±0.56$^{a}$ | 100 |
| Fe-NPs$_{50}$ | 5.25±0.06$^{a}$ | 6.87±0.24$^{d}$ | 1.61±0.30$^{d}$ | 30.75±6.01$^{d}$ | 0.19±0.04$^{d}$ | 1.57±0.23$^{b}$ | 100 |

Values with different superscript letter in the same column indicate significant difference at $P< 0.05$.
IW = initial weight (g), FW = final weight (g), WG = weight gain (g), %WG = percentage weight gain, SGR = specific growth rate (%/day), CF = condition factor

As shown in Table 4, FCR of fish decreased as dietary Fe-NPs increased up to 40 mg/kg feed of Fe-NPs compared to other groups. Increase in Fe-NPs concentration in feed increased the feed efficiency up to Fe-NPs$_{40}$ group and further increase in concentration of Fe-NPs up to 50 mg/kg feed of Fe-NPs reduced the FCE. PER and PPV were also found to show their better performance at Fe-NPs$_{40}$ group and again further increase in concentration reduced these values. However, fish group at Fe-NPs$_{50}$ was not significantly ($P > 0.05$) different from control group in case of FCE, PER and PPV.

**Table 4:** Feed utilization parameters of *Clarias batrachus* fed Fe-NPs enriched diets

| Treatments | FCR | FCE | PER | PPV (%) |
|---|---|---|---|---|
| Control | 11.96±3.00$^{a}$ | 0.09±0.03$^{d}$ | 0.26±0.08$^{d}$ | 5.54±0.53$^{d}$ |
| Fe-NPs$_{10}$ | 7.15±1.58b$^{c}$ | 0.14±0.04$^{c}$ | 0.43±0.10$^{c}$ | 7.34±0.92$^{c}$ |
| Fe-NPs$_{20}$ | 4.39±0.32c$^{d}$ | 0.23±0.02$^{b}$ | 0.68±0.05$^{b}$ | 10.28±0.42$^{b}$ |
| Fe-NPs$_{30}$ | 3.96±0.11$^{d}$ | 0.25±.01$^{b}$ | 0.75±0.02$^{b}$ | 10.54±0.24$^{b}$ |
| Fe-NPs$_{40}$ | 2.75±0.06$^{d}$ | 0.37±0.01$^{a}$ | 1.08±0.02$^{a}$ | 20.25±0.25$^{a}$ |
| Fe-NPs$_{50}$ | 10.04±2.20$^{b}$ | 0.10±0.02$^{d}$ | 0.31±0.06$^{d}$ | 6.19±0.66$^{d}$ |

Values with different superscript letter in the same column indicate significant difference at $P< 0.05$.
FCR = feed conversion ratio, FCE = feed conversion efficiency, PER = protein efficiency ratio, PPV = protein productive value (%)

The proximate composition of whole body is given in Table 5. All the proximate compositions were significantly ($P< 0.05$) affected by dietary Fe-NPs at different concentrations. The protein and lipid content of whole bodies were significantly increased up to the fish group of Fe-NPs$_{40}$; afterwards a sudden decrease was occurred at Fe-NPs$_{50}$ fish group, but the lipid content was not significant differ among control, Fe-NPs$_{10}$, Fe-NPs$_{20}$ and Fe-NPs$_{50}$ fish group. The ash and moisture content were significantly affected by increase in concentrations of Fe-NPs in diets compared to control group. Ash content was not found to differ significantly among Fe-NPs$_{10}$, Fe-NPs$_{20}$ and Fe-NPs$_{30}$ fish groups, whereas moisture content was not significantly different between Fe-NPs$_{20}$ and Fe-NPs$_{30}$ fish groups.

**Table 5:** Muscle composition of *Clarias batrachus* fed Fe-NPs enriched diets

| Treatments | Protein (%) | Lipid (%) | Ash (%) | Moisture (%) |
|---|---|---|---|---|
| Control | 8.75±0.01$^{e}$ | 2.00±0.02$^{c}$ | 0.31±0.01$^{d}$ | 80.44±0.02$^{e}$ |
| Fe-NPs$_{10}$ | 8.93±0.02$^{d}$ | 2.01±0.01$^{c}$ | 0.35±0.02$^{c}$ | 83.30±0.01$^{d}$ |
| Fe-NPs$_{20}$ | 9.32±0.02$^{b}$ | 2.00±0.02$^{c}$ | 0.37±0.02$^{c}$ | 83.70±0.04$^{c}$ |
| Fe-NPs$_{30}$ | 9.09±0.06$^{c}$ | 2.09±0.02$^{b}$ | 0.37±0.02$^{c}$ | 83.72±0.01$^{c}$ |
| Fe-NPs$_{40}$ | 12.31±0.02$^{a}$ | 2.15±0.02$^{a}$ | 0.44±0.03$^{b}$ | 84.34±0.02$^{b}$ |
| Fe-NPs$_{50}$ | 8.91±0.04$^{d}$ | 2.02±0.01$^{c}$ | 0.52±0.01$^{a}$ | 85.67±0.02$^{a}$ |

Values with different superscript letter in the same column indicate significant difference at $P< 0.05$.

Hematological changes in the plasma of *C. batrachus* fed the diets containing various Fe-NPs concentrations are presented in Table 6. Total protein, cholesterol and triglyceride of the plasma of fish were found affected by the addition of Fe-NPs in feed and increased up to a concentration of 40 mg/kg feed of Fe-NPs. Whereas, further increase in concentration up to 50 mg/kg feed of Fe-NPs significantly reduced the value of these parameters compared to Fe-NPs$_{40}$ fish group. High density lipoprotein (HDL) and low density lipoprotein (LDL) were also found to influence by the supplementation of Fe-NPs. However, HDL showed an increasing trend in its value in order of control > Fe-NPs$_{10}$ > Fe-NPs$_{20}$ > Fe-NPs$_{30}$ > Fe-NPs$_{40}$ > Fe-NPs$_{50}$ and LDL showed decreasing trend as opposite to the direction of HDL. Blood enzyme profile of *C. batrachus* fed Fe-NPs enriched diets are shown in Table 5. Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were significantly increased with increasing the concentration of Fe-NPs compared to control group.

**Table 6:** Serum biochemical profile of *Clarias batrachus* fed Fe-NPs enriched diets.

| Parameters | Control | Fe-NPs$_{10}$ | Fe-NPs$_{20}$ | Fe-NPs$_{30}$ | Fe-NPs$_{40}$ | Fe-NPs$_{50}$ |
|---|---|---|---|---|---|---|
| Total protein (%) | 12.59±0.02$^{f}$ | 12.78±0.02$^{e}$ | 13.58±0.02$^{c}$ | 14.25±0.02$^{b}$ | 15.14±0.02$^{a}$ | 13.47±0.02$^{d}$ |
| Cholesterol (g/dl) | 205.12±0.56$^{f}$ | 209.67±0.02$^{e}$ | 213.43±0.02$^{d}$ | 215.75±0.04$^{c}$ | 219.15±0.02$^{a}$ | 217.33±0.02$^{b}$ |
| Triglyceride (g/dl) | 151.05±0.03$^{f}$ | 156.05±0.01$^{e}$ | 160.33±0.02$^{d}$ | 161.56±0.02$^{b}$ | 167.53±0.01$^{a}$ | 160.97±0.02$^{c}$ |
| HDL (g/dl) | 51.36±0.02$^{f}$ | 52.46±0.01$^{e}$ | 53.97±0.02$^{d}$ | 54.17±0.02$^{c}$ | 54.37±0.02$^{b}$ | 55.14±0.02$^{a}$ |
| LDL (g/dl) | 147.59±0.02$^{a}$ | 143.31±0.02$^{b}$ | 142.17±0.02$^{c}$ | 141.39±0.02$^{d}$ | 140.55±0.02$^{e}$ | 140.28±0.03$^{f}$ |
| ALT (U/L) | 31.25±0.02$^{f}$ | 31.37±0.02$^{e}$ | 32.18±0.02$^{d}$ | 32.53±0.02$^{c}$ | 32.58±0.02$^{b}$ | 32.78±0.02$^{a}$ |
| AST (U/L) | 35.36±0.01$^{f}$ | 35.84±0.02$^{e}$ | 35.87±0.02$^{d}$ | 36.15±0.02$^{c}$ | 37.26±0.02$^{b}$ | 37.34±0.02$^{a}$ |
| ALP (mg/dl) | 13.33±0.02$^{f}$ | 13.64±0.01$^{e}$ | 14.08±0.01$^{d}$ | 14.17±0.02$^{c}$ | 14.25±0.02$^{b}$ | 14.33±0.02$^{a}$ |

Values with different superscript letter in the same row indicate significant difference at $P< 0.05$.
HDL = high density lipoprotein, LDL = low density lipoprotein, ALT = alanine aminotransferase, AST = aspartate aminotransferase,

ALP = Alkaline phosphatase

Fe concentration in muscle, liver and serum of *C. batrachus* fed the experimental diets for 30 days was generally dose dependent, increasing with increase in dietary Fe-NPs concentration (Figure 1). Fe accumulated mostly in the serum followed by the muscle and liver in order of serum > muscle > liver. Therefore, serum *C. batrachus* is a more important storage site than other tissues. Fe contents of fish fed Fe-$NPs_{50}$ diets were significantly higher than those of fish fed Control, Fe-$NPs_{10}$, Fe-$NPs_{20}$, Fe-$NPs_{30}$ and Fe-$NPs_{40}$. Liver Fe content of fish fed Fe-$NPs_{30}$ and Fe-$NPs_{40}$ were not significantly different and the highest Fe content was found in fish group fed Fe-$NPs_{50}$ diet. Similar to muscle Fe content, serum Fe content of Fe-$NPs_{50}$ diets fed fish was also significantly higher than those of fishes fed Control, Fe-$NPs_{10}$, Fe-$NPs_{20}$, Fe-$NPs_{30}$ and Fe-$NPs_{40}$ diets.

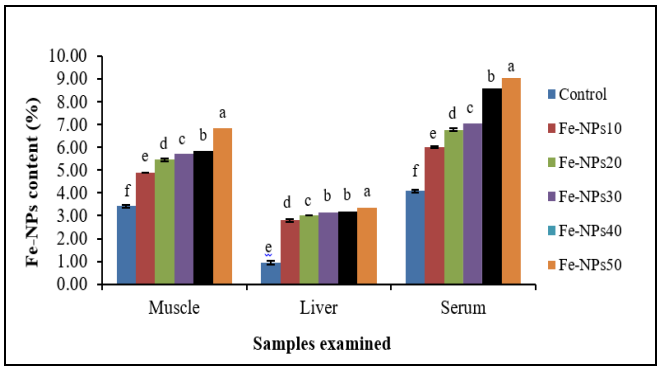


**Fig 1:** Bioaccumulation of Fe-NPs in muscle, liver and serum of *Clarias batrachus*. Column with different letters in each sample indicates significant difference ($P < 0.05$).

## Discussion

In the present study, there is no obvious effect of the Fe-NPs added to feeds on water quality. No mortality was also observed throughout the feeding trial experiment. This revealed that rearing condition was favorable during the experiment.

During the present study, Fe-NPs supplementation at different concentrations in the experimental diets resulted in higher growth performance as compared to the control diet in a dose dependent manner. Similar observation was also made by Behera *et al.* [5] in *Labeo rohita*, where they showed that iron-supplementation in diet improved the growth performance of fish. Significantly higher ($P<0.05$) FW, WG, %WG, SGR and CF were noted for the fish group fed 40 mg/kg feed of Fe-NPs. In the studies conducted by Gatlin and Wilson [15]; Lim *et al.* [16] and Sealey *et al.* [17] showed the total dietary Fe requirement for optimum growth, feed efficiency, hematological values and immune response of juvenile channel catfish was about 30 mg/kg diet, which was lower than the dose used in the present study. However, further increase in concentration of Fe-NPs up to 50 mg/kg feed significantly reduced the growth performance of *C. batrachus* in the present study might be due to the toxicity caused by higher Fe-NPs uptake. Similar results have also been found in other studies, suggesting that increasing the level of iron supplementation up to supra-optimal concentration have deleterious effect on living animals [18, 19].

Feed utilization parameters have close relationship with growth rate. In the present study, FCR decreased with increase in dietary Fe-NPs up to a certain level of 40 mg/kg feed of Fe-NPs indicates the superior quality of this dose over the control group and other doses. On the contrary, FCE, PER and PPV were found to increase with increasing the Fe-NPs in diet up to the same concentration. Thus, Fe-NPs toxicity at higher doses (50 mg/kg feed of Fe-NPs) would not only influence the growth performance of fish but also feed utilization parameters. Similarly, impaired feed conversion in channel catfish and juvenile yellow catfish at high levels of copper has been reported by Tan *et al.* [20].

Concentration-based increases in muscle biochemical compositions, such as protein and lipid compared to control group suggests that dietary Fe-NPs has influence on nutrient absorption and enhances the synthesis and storage of protein and lipid in *C. batrachus*. In the present study, the higher levels of protein and lipid recorded in 40 mg/kg feed of Fe-NPs supplemented feed fed the fishes suggest maximum influence of Fe-NPs on the metabolism of *C. batrachus*. At the higher doses (50 mg/kg feed of Fe-NPs) protein level of muscle was gradually decreased. The present observation is in line with the previous findings [21, 22] as the total protein content decreased in muscle of the experimental animals at higher doses of NPs due to overutilization of protein on overcoming stress. During the study period, moisture and ash contents of fish muscle were found positively correlated with dietary Fe-NPs concentrations and significantly higher moisture and ash content were noted for the fish group fed Fe-NPs50 diet. Similar observation was also made by Mohseni *et al.* [23], where they showed that increase in NPs concentration increases the moisture and ash content of fish muscle.

Blood parameters are one of the important indicators for monitoring health condition of fish in terms of physiological as well as the pathological point of view [24, 25]. In this study, effect of Fe-NPs in blood parameters was increased in a dose-dependent manner. Significantly higher total protein, cholesterol and triglyceride were found in fish group fed 40 mg/kg feed of Fe-NPs indicating superior health condition of this fish group as Riche [26] reported that serum total protein is a broad clinical indicator of health, immune competence, stress, and nutritional condition in fish. But at the dose of 50 mg/kg feed of Fe-NPs the value of above mentioned blood parameters were significantly reduced. The decrease in serum total protein may be due to increased lipolysis [27] and detoxification mechanism during stress [28] caused by toxic effect of Fe-NPs at higher dose. These results were also similar to those of Zaghloul *et al.* [29] who studied the effect of copper toxicity on *C. gariepinus*. The increased level of serum HDL in the present study was accompanied with decreased concentrations of LDL.

Serum enzymes such as AST, ALT and ALP could be used as sensitive biomarkers in ecotoxicology, because they provided an early warning of potentially hazardous alterations in contaminated aquatic organisms [30, 31, 32]. Significant increase in the activities of serum ALP, AST and ALT is considered as the response of organism to stressors [33].The results in the present study indicated a significant increase in serum enzyme (AST, ALT and ALP) activities, when *C. batrachus* were exposed to the Fe-NPs enriched diets compared to control group. These results were in agreement with Zaghloul *et al.* [26] who studied the effect of copper toxicity on *C. gariepinus* and showed a significant increase in serum enzyme (AST, ALT and ALP) activities in comparison to the control group. Therefore, increase in AST, ALT and ALP activities in serum of *C. batrachus* was assumed to be a result of liver damage by high Fe-NPs content at Fe-$NPs_{50}$ fish group.

During the present experiment, the highest accumulation of Fe was observed for the fish group fed Fe-$NPs_{50}$ diet and among the samples (muscle, liver and serum) higher accumulation was occurred in serum of *C. batrachus*. It might be due to the function of Fe entering and leaving the blood stream. Similar observation was also made by Silva *et al.* [34]. Higher Fe accumulation at the serum of fish group fed Fe-NPs indicates higher absorption and bioavailability of Fe by this fish group. Moreover, less absorption of Fe in fish feed control diet indicates deficiency of Fe and suggested that iron supplementation is necessary for *C. batrachus*.

**Conclusion**

In conclusion, the result of the present study demonstrate that the growth and feed utilization parameters of *C. batrachus* were optimal when the diet contained a Fe-NPs level of 40 mg/kg feed of Fe-NPs. The results are important as a basic knowledge in formulating cost-effective feeds for this catfish species.

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