Indian J. Anim. Nutr. 2024. 41 (2): 304-312
doi: 10.5958/2231-6744.2024.00039.0

Indian Journal of Animal Nutrition

Effect of Dietary Iron Levels on Growth, Body Composition and Enzyme Activities of Rohu (*LabeoRohita*Hamilton) Advanced Fingerlings

S. Chanda[1], B.N. Paul[1*], P.Singh[1], K.Ghosh[2] and S.S. Giri[4]
[1]Regional Research Centre,Central Institute of Freshwater Aquaculture
Rahara, Kolkata-700 118, West Bengal
[2]Aquaculture Laboratory, Department of Zoology, The University of Burdwan
Golapbagh, Burdwan- 713 104
[4]Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar- 751 002, Odisha
*Correspondence:bnpaulcifa@gmail.com

ABSTRACT

The iron requirement study was conducted in *Labeorohita* advanced fingerlings for 67-day. Casein, gelatin and dextrin-based six isonitrogenous purified diets were formulated with graded levels of iron (as $FeSO_4.7H_2O$) viz., Group 1 (0 ppm; Control), Group 2 (25 ppm), Group 3 (50 ppm), Group 4 (75 ppm), Group5 (100 ppm) and Group 6 (125 ppm). The results indicated that net weight gain, specific growth rate, daily growth coefficient and protein efficiency ratio were significantly ($P<0.05$) higher in group 3 (fed 50 ppm iron).The feed conversion ratio was significantly lower in group 3. The activities of aspartate transaminase, alanine transaminase and alkaline phosphatase weresignificantly ($P<0.05$) higher in group 3. The carcass composition of advanced rohu fingerlings revealed that carcass protein wassignificantly ($P<0.05$) higher in group 3 (50 ppm iron). The present study indicated that 50 ppm dietary iron is required for *Labeorohita* advanced fingerlings for optimum growth and tissue iron deposition.

KEY WORDS: AST, ALT, ALP, Growth performance and iron, *Labeorohita*

Article received: 22 May 2024; Article accepted: 07 July 2024

INTRODUCTION

Iron is an essential trace element required for haemoglobin myoglobin, cytochrome, peroxidase, catalase and many other enzymes that are mainly involved in oxygen transport, transformation, cellular respiration and lipid oxidation reaction, Shiau and Su. (2003), Ye et al.(2010), Ling et al.(2010).Regulatory effects of iron are also associated with hormone synthesis and fatty acid metabolism, Brody(1994). Iron deficiency causes immune suppression, growth depression, disease susceptibility, microcytic anaemia and poor food conversion, Anderson et al.(1996). Iron is thus essential and, in excess, can be toxic,Anderson et al.(1997). It is absorbed and transported in the body in a protein-bound form such as haem, Lovell(1989) and non-haem compounds such as transferrin, ferritin and flavin iron enzymes. Fish absorb Iron from water through the gill membrane, Lall (1989).However;it is still considered that Fe is dietary essential for the normal growth of fish, Chanda et al. (2015).

The factors that influence dietary mineral requirements of fish are species, life stage, sex, trophic level, mineral concentration in the culture system, salinity and temperature, Prabhu et al. (2014).The information on the Iron requirement of Indian fish species is not available except in our only study,rohu fry; Chanda et al. (2017). The present study was undertaken to determine the iron requirement of *L. rohita* advanced fingerlings for their growth, hepatic function and deposition at the tissue level.

MATERIALS AND METHODS

Experimental diet preparation

Six purified diet was prepared with graded iron levels at 25 ppm, 50 ppm, 75 ppm, 100 ppm and 125 ppm. The iron levels were adjusted with á-cellulose.

$FeSO_4.7H_2O$ was used as the iron source. Dextrin, gelatin, casein, and oils from plants and animal sourceswith vitamins and minerals were used as feed ingredients. The mineral (iron-free) mixture was prepared Ogino

(1979), and the vitamin mixture as per Halver and Hardy (2002). The ingredients were procured from Hi Media Laboratories, Mumbai, India. The purified diet was prepared as per Paul et al. (2006). All the ingredients except gelatin were properly mixed with oil. Gelatin was dissolved in boiling water and mixed with the mixture to form stiff. The resultant dough was pelleted with a medium-size meat grinder with a 2.0 mm diameter template. The moist strands so obtained were sealed and frozen at -20^{0}C.The strands were broken into appropriate sizes before feeding the fish. Diet ingredients and proximate composition are presented in Tables 1 and 2, respectively.

Experimental Design

The experiment was conducted in Rohu (*Labeorohita*) advanced fingerlings with an initial average weight (24.36 ±0.44 g). The fish were collected from the fish farm of RRC, ICAR-CIFA, Rahara, West Bengal. Twelve rohu fishes were randomly stocked in each ofthe 18Fibre Reinforced Plastic tanks. The tanks were plumbed with a continuous water flow-through system, and the water flow rate was maintained at 1.5L/h. Initially, the control dietwas fed to stocked fish for one week to acclimateto the system. After acclimatisation, the fish were fed twice daily *ad libtium,* Paul et al.(2004) every dayfor 67 days with the formulated feeds to triplicate treatment tanks. The tanks were checked routinely for mortality and physical and behavioural changes, if any. Stored groundwater (Fe level>0.5ppm) was used to rear experimental fishes. Fortnightly sampling was done to assess the growth of the fish.

Analysis

Fortnightly the fish were weighed, and their mortality was recorded. At the end of the feeding trial, fish were collected from each tank; individual body weight was weighed and analysedto calculate the growth parameters. Net weight gain (NWG), feed conversion ratio (FCR) and specific growth rate (SGR) was calculated as per Paul and Giri (2015). The Daily growth coefficient (DGC)was calculated as per Cowey (1992).

Estimation of metabolic enzymes

Fish were anaesthetised and sacrificed with clove oil (50 µl/L water). Six fishes from each treatment were randomly sampled for the liver functioning test (LFT) by the assay of liver marker enzymes (Table No. 4). The blood samples, after proper anaesthesia,were collected directly from the heart using a 3 ml medical syringe pre-rinsed with anticoagulant. The collected blood was then centrifuged at 3000rpm to separate plasma, which is required for in vitro enzyme study by 2,4 dinitrophenylhydrazine following the Reitman and Frankel (1957) using standard kits.

Proximate composition of experimental diets and fish carcass

Proximate compositions of the experimental diets and fish carcasses wereanalysed as per AOAC (2005). Oven drying determined moisture content (initially at 100^{o}C, then at 60^{o}C). Crude protein was analyzed by the Kjeldahl method (Kjeldahl nitrogen*6.25), crude fat was estimated by extracting the samples with solvent for 2-4 h using a Socs plus (Pelican equipment, Chennai, India) and total ash was estimated by the combustion of samples into the Muffle Furnace at 600^{0}C for about 6hour for feed and carcass tissues. The ash samples from carcass tissue were acid digested according to AOAC (2005) method to determine the iron concentration. Iron content in all samples including water was estimated usinga flame atomic absorption spectrophotometer (Thermo Fisher, M Series, U.K)' Paul et al. (2014) following digestion of samples (Exclude water) in acid. After digestion, the Kjeldahl flasks were cooled and the mineralized samples were diluted to 100 ml with double distilled water.The water quality parameters were assessed as per APHA (2005).

Statistical analysis

The data were statistically analyzed as per Snedecor and Cochran (1994) by one-way ANOVA to calculate the dietary effect of iron level on growth performance and activities of fish metabolic enzymes. The least significant difference was used to compare the mean values. Data are represented as treatment mean (±) standard deviation of the mean (SD).

RESULTS AND DISCUSSION

The ingredient composition of the diets used in 6 groups has been presented in Table 1. Proximate compositions of the formulated feeds are shown in Table 2. The protein content of all the dietary treatments ranged from 29.52 to 32.52 (%).

Table 1. Feed Formulation (% Dry matter basis)

Particulars	Group 1 Control	Group 2 (25 ppm)	Group 3 (50ppm)	Group 4 (75ppm)	Group 5 (100ppm)	Group 6 (125ppm)
Casein	32	32	32	32	32	32
Gelatin	9	9	9	9	9	9
Dextrin	32	32	32	32	32	32
CMC	2	2	2	2	2	2
α-Cellulose	13	12.9	12.9	12.9	12.9	12.9
Sunflower oil	3	3	3	3	3	3
Cod liver oil	3	3	3	3	3	3
Vitamin mixture[1]	1	1	1	1	1	1
Mineral mixture[2]	5	5	5	5	5	5
Fe source (Ferrous sulphate)	0	0.0025	0.0050	0.0075	0.0100	0.0125

[1]Halver and Hardy (2002);
Vitamin (g): Thiamine hydrochloride (0.5); Riboflavin (0.5); Calcium pantothenate (1.0); Nicotinic acid (0.605); Biotin (0.003); Pyridoxine hydrochloride (0.0825); Folic acid (0.15); Inositol (20); L-Ascorbyl 2-Monophosphate (0.202); Choline chloride (4.4); Menadione (0.4); Retinol acetate (0.04); Cholecalciferol (0.000469)
[2]Ogino (1979)
Mineral (g): NaCl (1.0); MgSO4.7H_2O(15.0); NaH_2PO_4.2H_2O(25.0); KH_2PO_4(32); $Ca(H_2PO_4)2H_2O$(20); Ca Lactate (3.5); Cellulose (2.5); Trace Minerals (1.0)

Fat and ash content ranged from5.20-5.78 (%) and13.91-15.24 (%) respectively. The dry matter, crude protein, fat and ash content levels were similar in all the treatment groups.

Table 2. Proximate Composition of Feed (% w/w basis)

Particulars	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Dry matter	79.0±0.09	78.7±0.25	78.5±0.11	78.9±0.53	78.6±0.34	78.6±0.02
Protein	31.2±0.82	30.7±0.28	30.6±0.02	32.5±0.31	29.5±0.34	29.9±0.07
Fat	5.34±0.06	5.20±0.05	5.71±0.10	5.78±0.27	5.25±0.07	5.49±0.03
Ash	14.2±0.02	14.6±0.43	14.7±0.26	15.1±0.07	13.9±0.27	15.2±0.28

Data are expressed as Mean ± SD.

There was no adverse effect of different iron sources on water quality. Temperature of the water was22.4±0.25^{0}C and dissolved oxygen was6.80±0.16 mg/l, total hardness level was 103.40±0.12 mg/l and total alkalinity and pH were in the range of 107.36±0.65 mg/l and 7.6±0.73 respectively. The water quality parameters were within the normal range for fish production (Banerjee, 1967) indicating that the fish were not under stress. The iron concentration of rearing water was ranged between 0.04-0.08 mg/l throughout the trial period.

Growth performances

Initial weight, final weight, net weight gain (NWG), feed conversion ratio (FCR) and specific growth rate of fish fed different dietary iron levels are illustrated in Table3. The initial weight of the fish ranged from 23.92 to 24.78(g).

Table 3. Growth performance of rohu fingerlings fed with different rations

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Initial wt.	24.1±0.83	24.4±0.55	24.1±0.83	24.8±0.34	24.16±0.28	23.9±0.04
Final wt.	26.02^{b}±0.95	26.1^{b}±0.74	28.2^{c}±0.25	25.8^{b}±0.46	24.49^{a}±0.35	24.14^{a}±0.16
NWG (g/67d)	1.86^{c}±0.12	1.68 ^{c}±0.19	4.08^{d}±0.58	1.01^{b}±0.12	0.33^{a}±0.07	0.26 ^{a}±0.11
FCR	2.66^{c}±0.145	2.65b ±0.73	2.58 ^{a}±0.37	2.61 ^{ab}±1.04	4.60 ^{d}±0.37	6.80 ^{e}±0.80
SGR	0.12^{c}±0.15	0.11 ^{c}±0.01	0.22^{d}±0.45	0.65^{b}±0.005	0.02^{a}±0.005	0.012^{a}±0.007
PER	1.24^{c}±0.12	1.33^{c}±0.05	1.37^{d}±0.06	1.17^{c}±0.04	0.77^{b}±0.07	0.56^{a}±0.03
DGC	1.02^{c}±0.05	0.94^{c}±0.06	2.01^{d}±0.02	0.57^{b}±0.03	0.19^{a}±0.02	0.22^{a}±0.02

Data are expressed as Mean ± SD. Values with different superscript ($p< 0.05$) in a row differ significantly.

The NWG and SGR significantly improved ($P<0.05$) with increasing levels of dietary iron up to 50ppm. Further increase in dietary iron concentration led to decrease in above growth parameters. The value of the FCR was significantly ($P>0.05$) lower for Group containing 50ppm iron. Survivability per cent of the rohu advanced fingerlings were 85% during the experimental period. In this investigation, PER of the fish attained its maximum at 50 ppm dietary iron and then remained significantly decreased with higher iron levels.

The liver enzyme contents of the experimental fish arepresented in Table no. 4. AST content was significantly higher ($P<0.05$) in group 3 followed by group 4.

Table 4. Specific activity of metabolic enzymes of rohu fed varying levels of Dietary iron

Particulars	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
AST (IU/L)	19.9^{b}±0.06	20.62^{bc}±0.84	26.14^{d}±0.71	20.98^{c}±0.05	16.64^{a}±0.62	16.31^{a}±0.38
ALT (IU/L)	132.5^{b}±0.08	132.2^{b}±0.56	138.5^{d}±0.39	134.6^{c}±0.05	129.1^{a}±0.07	130.1^{a}±0.71
ALP (Å)	10.2^{a}±0.46	11.01^{b}±0.54	14.1^{d}±0.62	10.5^{a}±0.14	12.1^{c}±0.01	10.2^{a}±0.02

Data are expressed as Mean ± SD. Values with different superscript ($P< 0.05$) in a row differ significantly.

In the case of ALT and ALP, values were also significantly ($P<0.05$) higher in group 3 compared to others.

Body composition and tissue iron concentration

Carcass body composition and tissue iron data in fish fed varying levels of dietary iron are depicted

in Table no. 5. Body moisture content varied from 76.42 to 77.19, protein ranged from 10.21 to 11.86(%), fat ranged from 1.82 to 2.40 (%) and total ash ranged from 4.48 to 5.18 (%).

Table 5. Carcass proximate composition (%w/w basis) and Fe tissue content of rohu after 67 d

Particulars	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Moisture	76.4±0.64	76.5±0.18	77.19±0.46	76.9±0.81	77.19±0.09	76.4±0.18
Protein	10.2±0.03^{a}	11.1±0.61^{c}	11.8±0.54^{c}	10.5±0.72^{a}	10.9±0.68^{b}	10.6±0.96^{a}
Fat	2.01±0.56	2.13±0.12	2.40±0.31	1.82±0.54	2.21±0.51	2.32±0.46
Ash	4.96±0.01	4.48±0.16	5.02±0.45	5.16±0.49	5.18±0.32	4.94±0.30
Tissue Fe content (ppm)						
Fe content	1.62±0.20^{a}	2.14±0.02^{b}	4.14±0.15^{d}	3.79±0.66^{c}	4.18±0.07^{d}	3.98±0.12^{c}

Data are expressed as Mean ± SD. Values with different superscript (P< 0.05) in a row differ significantly.

In the present study, the higher protein levels recorded in dietary iron supplemented Group 3with higher growth rate, which is in agreement with the earlier report,Chavez-Sanchez et al. (2000). However, varying dietary iron levels had no significant (P<0.05) effect on the moisture, fat and ash content of rohu.Whole body iron concentration was significantly (P<0.05) higher in Group 3 and 5. However the iron content in control and 25 ppm is in agreement with the earlier report, Paul etal.(2026)

Iron is the most investigated essential trace element, generally present in all body cells of vertebrates; Paul and Giri (2015). It is necessary to function several biochemical processes, including the electron transfer reaction, gene regulation, binding and transport of oxygen and regulation of cell growth. The presently reported study revealed that an increase in the dietary iron level up to 50ppm was associated with maximum gain and increased SGR of the *Labeorohita* advanced fingerlings. Similarly, the lowest FCR and the maximum PER values were recorded in the rohu fed diets with 50ppm iron level. Our result are in agreement with the earlier reports indicating that the iron requirement ranges from 30 to 170 mg/kg in salmon, channel catfish, sea bream and eel; Chanda et al.(2015). NRC (2011) indicated that the dietary requirement of iron for fish ranged from 30 to 150 mg/kg. The required iron level detected in the present study was lower than the suggested iron levels documented for other fishes. Earlier study reported thatdietary iron requirement required for gold fish was 139.06ppm, Das et al. (2013), 80ppm for guppy, Shim and Ong (1992); and 85 ppm for hybrid tilapia, Shiau and Su (2003),300ppm for juvenile grass carp, Suet al. (2007),245 ppm for grouper,Ye et al. (2007) when $FeSo_4$ used as an iron source. However, tilapia requires much more dietary iron (150-160 ppm Fe kg-1 diet) when fed with ferric citrate supplemented diets, Shiau and Su. (2003). A 199 ppm Fe/kg diet is required to prevent iron deficiency in common carp when ferric chloride is used as an iron source. Sakamoto and Yone (1978a). Our results agree with earlier reports indicating that diet containing 50 ppm of iron and other purified feed ingredients shows better net weight gain in rohu advanced fingerlings. Iron requirement of several fish species showed that 58.8-166.4 mg/kg, Antony et al. (2013). Chanda et al.(2017) reported that 50ppm supplementation of Fe with purified diet showed better performance in rohu fingerlings.A lower requirement of dietary iron in the present study to prevent iron deficiency symptoms may be because the ferrous iron is absorbed more efficiently than the ferric iron. However, comparing with the dietary iron requirement reported for juvenile gibel carp (202mg/kg diet) and red sea bream (150mg/kg diet), Sakamoto and Yone. (1978b) the present estimated dietary iron requirement values of 50 ppm is lower but higher than that determined for *P. vannamei*s

12mg/kg diet, Davis et al. (1992); channel catfish is 30mg/kg, Gatlin and Wilson (1986) and Bjamevik and Maage (1993) studied that the iron requirement for Atlantic salmon is more than 33mg/kg dry diet. However, the difference of dietary iron requirement differs between species or even within the same species due to differences in body iron demand, presence of soluble iron in rearing water, feeding efficiency, iron source used, growth rate, life history stage, food sourceas reported by Shearer (1995).

The present study appraised activities of some metabolic enzymes to evaluate the effects of varying iron levels. The fish liver is the hotspot for Trans deamination with ALT and AST as the major enzymes, Enes et al. (2006), Kumar et al. (2008). Serum enzymes such as AST,ALT and ALP could be used as sensitive biomarkers in ecotoxicology because they provide an early warning of potentially hazardous alterations in contaminated aquatic organisms, Nel et al.(2009). Significant increase in serum AST and ALT activities is the organism's response to stressors, Bitiren et al. (2004). The result in the present study indicated a significant increase in serum enzyme activities, when *L. rohita* were exposed to the ironenriched diets. These results agreed with Zaghloul et al. (2006). Hence, the present study concluded that the treated fish contains an optimum range of liver marker enzymes (ALT, AST and ALP) which were significantly ($P<0.05$) higher in Group 3 indicating the better health conditions of the group.

Deficiency and surplus regarding mineral utilization in fish is a delicate balance; excess intake may cause toxicity, Lall and Milley (2008). The Report is scarce concerning the nutritional deficiencies due to inadequate dietary iron in Indian Major Carps. However, there are some reports that dietary iron deficiency induces anaemia in brook trout, *Salvelinusfontinalis*.Kawatsu (1972); Yellow tail, *Seriolaquinqueradiata,*Ikeda et al. (1973); eels (*Anguilla japonica*), Nose and Arai (1979); red sea bream, *Pagnus major,* Sakamoto and Yone (1976); Atlantic salmon, *Salmosalar,* Anderson et al.(1996) and common carp, (*Cyprinuscarpio*) Sakamoto and Yone (1978b). In Channel catfish (*Ictaluruspunctatus*) iron deficiency suppressed haematocrit, haemoglobin and plasma iron levels and caused transferring saturation, Gatlin and Wilson (1986). The present experiment is mainly based on the minimal inclusion of dietary iron in fish regarding its optimum growth and clinical deficiency. Fish fed with diet containing more than 75 ppm shows decrease in growth which may be due to higher iron concentration, Kanazawa et al.(1984). Iron deficiency is known to cause yellowish white liver condition in carp, Sakamoto and Yone. (1978b). Poor feed utilization and lowering of plasma iron level and transferring situation is noted in channel catfish, Gatling and Wilson (1986). The hatching rate of rainbow trout eggs was poor when iron content was low in feed, Desjardins(1985).

CONCLUSION

Results of the present study indicatedthatsupplementing 50 ppm of iron in purified diets significantly improved growth, nutrient utilization, carcass proximate compositions, serum enzyme content and tissue iron concentration in *Labeorohita* advanced fingerlings.The result would help in formulating mineral mixture for the Indian major carps.

ACKNOWLEDGEMENT

The authors greatly acknowledge the help and support of the Director of ICAR-Central Institute of Freshwater Aquaculture, Bhubaneswar and the Head, Department of Zoology, University of Burdwan, for providing the necessary facilities to experiment.

REFERENCES

Antony, J.P, P., Scharma, J.W. and Kaushik, S.J. 2013. Quantifying the dietary phosphorus requirement of fish-a metaanalytic approach. Aquaculture Nutrition. 19:243-249.

Anderson,F., Lorentzen, M., Waagbo,R. and Maage,A. 1997.Bioavailability and interactions with other micronutrients of three dietary iron sources in Atlantic salmon, *Salmo salar*, smolts. Aquaculture Nutrition. 3: 239-246.

Anderson, F., Maage, A. and Julshamn,K. 1996. An estimation of dietary iron requirement of Atlantic salmon, *Salmo salar* L.,parr. Aquaculture Nutrition. 2: 41-47.

AOAC. 2005. *Official methods of Analysis*, 18thEdn. Association of Official Analytical Chemists, Virginia, U.S.A.

APHA. 2005. *Standard Methods for Examination of Water and Wastewater*. 21st Edn. American Public Health Association, Washington, D.C., U.S.A.

Banerjee,S.M. 1967. Water Quality and Soil Condition of Fishponds in Some States of India in Relation to Fish Production. India Journal of Fisheries, 14 : 115-144

Bitiren, M.,Karakilcik, A.Z., Zerin,M.,Aksoy, N. and Musa, D. 2004. Effects of selenium on histopathological and enzymatic changes in experimental liver injury of rats. Experimental and Toxicological Pathology. 56: 59-64.

Bjamevik, M. and Maage, A. 1993. Effects of dietary iron supplementation on tissue iron concentration and haematology in Atlantic salmon (*Salmosalar*). Fisker Direktoratets Skrifter Serie Ernearing. 6: 35-45.

Brody,T. 1994. Nutritional Biochemistry. Academic Press, San Diego,U.S.A.

Chanda, S., Samanta, A., Paul B.N., Ghosh, K. and Giri, S.S. 2017. Effect of dietary iron level on Growth performance and Enzyme activity in Rohu (*Labeorohita* Hamilton) Fingerlings. Indian Journal of Animal Nutrition. 34: 224-228.

Chanda, S., Paul, B.N., Ghosh, K. and Giri, S.S. 2015. Dietary essentiality of trace minerals in aquaculture: A Review. Agricultural Reviews. 36: 100-112.

Chavez-Sanchez, C., Martinez-Palacios, C.A., Martinez-Perez, G. and Ross, L.G. 2000. Phosphorus and calcium requirements in the diet of American cichlid *Cichlasomauropthalmus*(Gunther). Aquaculture Nutrition. 6:1-9.

Cowey, C.B. 1992. Nutrition: Estimating requirements of rainbowtrout.Aquaculture.100: 177-189.

Das, A., Prakash,C., Babu, P.P.S., Sharma, A., Chanu,T. I.,Paul, L. and Verma, A.K. 2013. Dietary iron requirement of goldfish,*Carassiusauratus*. The Israeli Journal of Aquaculture-Bamidgeh. 66: 942-949.

Davis, D.A. and Lawrence, A.L. 1992. Evaluation of the Dietary Iron Requirement of *Penaeusvennamei*. Journal of World Aquaculture. 23: 15-22.

Desjardins, L.M. 1985. The effect of iron supplementation on diet rancidity and on the growth and physiological response of rainbow trout. M.Sc. Thesis, The University of Guelph, Ontario, Canada.

Enes, P., Panserat, S., Kaushik, S. and Oliva-Teles, A. 2006. Effect ofnormal and waxy maize starch on growth, food utilization and hepatic glucose metabolism in European seabass (*Dicentrarchuslabrax*) juveniles. Comparative Biochemistry and Physiology Part A. 143: 89-96.

Gatlin, D.M. and Wilson, R.P. 1986. Dietary copper requirement of fingerling channel catfish. Aquaculture.54: 277-285.

Halver, J.E. and Hardy, R.W. 2002. Fish Nutrition. 3rdEdn. Academic Press,U.S.A.

Ikeda, Y., Ozaki, H. and Vematsu, K., 1973. Effect of enriched diet with iron in culture of yellowtail. Journal of Tokyo University of Fisheries. 59: 91-99.

Kanazawa, A., Teshima, S. and Sasaki, M. 1984. Requirements of juvenile prawn for calcium, phosphorus, magnesium, potassium, copper, manganese and iron. Memoirs of the Facultyof Fisheries, Kagoshima University. 33: 63-71.

Kawatsu, H. 1972. Studies on the anemia of fish. V. Dietary iron deficient anemia in brook trout, *Salvelinusfontinalis*. Bulletin of Freshwater FisheriesResearch Laboratory. 22: 59-67.

Kumar, V., Sahu, N.P., Pal, A.K. and Gupta, S.K.

2008. Gelatinized to non-gelatinized starch ratio in the diet of *Labeorohita*: Effect on digestive and metabolic response and on growth. Journal of Animal Physiology and Animal Nutrition. 92(4): 492-501.

Lall, S. and Milley, J. 2008. Trace mineral requirements in fish and crustaceans. Trace elements in AnimalProduction System. 203.

Lall, S. 1989. The Minerals. In Halver, J.E. (Ed.), Fish Nutrition.2ndEdn, Academic Press Inc., New York, pp: 219-257.

Lovell, T. 1989. Nutrition and Feeding of fish. Van Nostrand Reinhold,New York.

Nel, A.E., Madler, L., Velegol,D., Xia, T., Hoek, E.M. and Somasundaran, P. 2009. Understanding biophysicochemical interactions at the nano bio interface. Nature Materials. 8: 543-557.

Nose, T. and Arai, S. 1979. Recent advances on studies on mineral nutrition of fish in Japan. In: T.V.R. Pillay and A.Dill (Eds., Advances in Aquaculture,pp.580-590. Faraham, England: Fishing News Book).

NRC. 2011. Nutrient Requirements of Fish. National Research Council, The National Academics Press, Washington, D.C.,U.S.A.

Ogino, C. and Takeda, H. 1979. The present situation of studies on fish nutrition. Proc. 7th Japan-Soviet Joint Symposium. Aquaculture. Tokyo. pp. 11-18.

Paul, B.N.,Sarkar,S., Giri,S.S., Rangacharyulu, P.V. and Mohanty, S.N. 2004. Phosphorus requirement and optimal Calcium/ Phosphorus ratio in the diet of mrigal*Cirrhinusmrigala*(Ham.) fingerlings. Journal of Applied Ichthyology.20 (4): 306-309.

Paul, B. N. and Giri, S.S. 2015. Freshwater Aquaculture Nutrition Research in India. Indian Journal of Animal Nutrition. 32:113-125.

Paul, B.N.,Sarkar,S., Giri,S.S., Mohanty, S.N. and Mukhopadhyay, P.K. 2006. Dietary Phosphorus and Calcium Requirements of Rohu*Labeorohita* Fry. Animal Nutrition and Feed Technology.6: 257-263.

Paul, B.N., Chanda, S., Sridhar, N., Saha, G.S. and Giri, S.S. 2016. Proximate, mineral and vitamin contents of Indian Major Carp. Indian Journal of Animal Nutrition.: 102-107.doi: 10.5958/2231-6744.2016.00018.9

Paul,B.N., Chanda, S., Das, S., Singh, P., Pandey, B.K., and Giri, S.S. 2014. Mineral assay in atomic absorption spectroscopy. Beats of Natural Sciences. 1:1-17.

Prabhu, A.J.P., Schrama, J.W. and Kaushik, S.J. 2014. Mineral requirements of fish: a systematic review. Reviews in Aquaculture. 6:1-48.

Reitman, S. and Frankel, S. 1957. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. American Journal of. Clinical pathology. 28: 56-63.

Roeder, M. and Roeder, R.H. 1966. Effect of iron on the growth rate of Fihes. The Journal of Nutrition. 90: 86-90.

Sakamoto, S. and Yone, Y. 1978a. Requirement of red seabream for dietary iron-II. Nippon Suisan Gakkaishi. 44: 223-225.

Sakamoto, S. and Yone, Y.1978b. Iron deficiency symptoms of carp. Nippon *Suisan Gakkaishi*. 44: 1157-1160.

Sakamoto, S. and Yone, Y. 1976. Requirement of red sea bream for dietary Fe-1 Rep. Fisheries ResearchLaboratory Kyushu University. 3: 53-58.

Shiau, S.Y. and Su, L.W. 2003. Ferric citrate is half as effective as ferrous sulfate in meeting the iron requirement of juvenile tilapia, *Oreochromisniloticus* x *O. aureus*. The Journal of Nutrition. 133: 483-488.

Shearer, K.D. 1995. Theuse of factorial modelling to determine the dietary requirements for essential elements in fishes. Aquaculture. 133: 57-72.

Shim, K.F. and Ong, S.I. 1992. Iron requirement

ofthe guppy (*Poecilia reticulate* Peters). Journal of FishAquactic Science. 6:33-40.

Snedecor, G.W. and Cochran, W.G. 1994. Statistical methods (8thEdn.). Oxford and IBH Publishing Co. Pvt.Ltd., New Delhi, India.

Su, C., Luo, L.,Wen, H., Sheng,X. and Li, S. 2007. Effects of dietary iron on growth Performance, Nutritional composition and some Blood indices of Grass carp (*Ctenopharyngodonidellus*). Freshwater Fish. 37: 48-52.

Watanabe, T., Kiron, V. and Satoh, S. 1997. Trace minerals in fish nutrition. Aquaculture.151: 185-207.

Zaghloul, K.H., Omar, W.A. and Abo-Hegab, S. 2006. Toxicity of copper in some freshwater. Egyptian Journal of Zoology. 47: 383 -400