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Optimization of Calcium and Phosphorus Levels in the Diet of *Cirrhinus mrigala* (Hamilton, 1822) Fingerlings

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ABSTRACT

An experiment was carried out to evaluate the optimum levels of calcium and phosphorus in the diet of mrigal, *Cirrhinus mrigala*. Ten treatments diets supplemented with additional calcium (Ca) and Phosphorus (P) in ratios 0:0 (C), 5.0:2.5 (D1), 5.0:3.75 (D2), 5.0:5.0 (D3), 7.5:2.5 (D4), 7.5:3.75 (D5), 7.5:5.0 (D6), 10:2 (D7), 10:3.75 (D8), 10:5.0 (D9), respectively for 90 days in triplicates were tested. Different concentrations of phosphorus as di-hydrogen-orthophosphate (0.25, 0.375 and 0.5 $100g^{-1}$ of diet) and calcium as calcium chloride (0.5, 0.75 and 1.0 g 100 g^{-1} of diet) were added to the basal diet. The Ca and P content of the holding water were 62.64±2.60 mg l^{-1} and 0.04±0.001 mg l^{-1}, respectively. Growth performance increased significantly ($P<0.05$) with 3.75 g kg^{-1} of phosphorus and 7.5 g kg^{-1} of calcium, but decreased beyond this concentration. Total ammonia excretion and reactive phosphate production was significantly higher in the fish of groups fed below and above this concentration. Fingerlings showed deficiency symptoms such as reduced growth, low intestinal enzymatic activity when fed on diets other than D5, clearly revealing that the concentration of 7.5 g kg^{-1} of calcium and 3.75 g kg^{-1} of phosphorus in the diet is optimum for enhancing growth in *C. mrigala*.

Keywords: Ammonia excretion, Calcium, *Cirrhinus mrigala*, Growth performance, Phosphorus

INTRODUCTION

Successful and sustainable aquaculture/fish culture is the need of time and it depends upon the provision of nutritionally adequate, environment friendly and economically viable supplementary compounded feeds. Growth, health and reproduction of fish are primarily dependent upon the adequate supply of nutrients both in terms of quantity and quality, irrespective of culture system in which they are grown. The natural feed has to be supplemented with balanced supplementary compounded diets for high survival and growth. The ultimate aim of feeding fish in aquaculture is to achieve maximum protein deposition with minimum inputs of feed at minimum cost. Fish nutrition is a challenging area of research and usual traditional fish diets i.e. rice bran, ground nut oil cake; do not contain enough nutrients required for good health. To reduce pollution and increase fish growth, a nutritionally balanced diet is necessary for improving not only fish growth but also to get fully nutritional fish flesh. Aquaculture is expanding in a period of environmental awareness and is, therefore, subject to regulations designed to limit its effect on the environment.

Feed containing primarily of plant ingredients are relatively low in available P (Cho and Bureau, 2001), therefore, the optimization of phosphorus required for fish growth is essential (Mgbenka and Vgwu, 2005). In order for world aquaculture production to continue to increase, it will be necessary to reduce discharge of wastes especially phosphorus (P), from fish farms to the aquatic environment. The ultimate source of P in aquaculture effluent is feeds. Any excess amount of P in diet above the optimum requirement for fish will be excreted by the fish. It is, therefore, critical to know precisely the dietary requirement of P in order to minimize excess P in diet without risking P deficiency in cultured fish. The dietary requirement of P has been studied for various fish and other animal species using fry or juveniles and indicators such as weight gain, feed efficiency level in various tissues, bone density, bone-breaking strength and enzyme activities (NRC, 1993). Calcium is an essential component constituent of all living cells involved in osmoregulation in some

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aquatic animals (Podoliak and Holden, 1966) and is known to be received from surrounding water and feed provided (Hossain and Furuichi, 2009). It has an interesting effect on the absorption or utilization of other divalent heavy metal by plants, fish and mammals as the toxicity of lead, copper, beryllium, cadmium and vanadium salts to fish becomes considerably less in the hard (calcium containing) waters than in soft or distilled water and deficiency may lead to skeletal deformities in fish larvae owing to the disruptions in developmental process (Cahu *et al.,* 2003).

The requirements of calcium and phosphorus for the fish should be considered together as the metabolism of two elements appears to be intimately connected; the greater part of the phosphorus of the body is associated with calcium in bones. Some information on the dietary requirements of calcium and phosphorus for optimum growth and metabolism in fish are available (Cowey, 1976). Hossain and Yoshimatsu (2014) have discussed the essentiality of optimum Ca:P and dietary Ca requirement in fishes. Keeping up a dietary Ca/P proportion should be considered, otherwise overabundance Ca or P may cause issues in the fish body or be remained unabsorbed and stacked in culture condition. Owing to the importance of calcium and phosphorus, the present study had been undertaken to provide an improved diet for the cultivation of a candidate fish species, *Cirrhinus mrigala.*

MATERIALS AND METHODS

The experiment was conducted under laboratory conditions at Aquaculture Research Unit of Kurukshetra University, Kurukshetra (25±1ºC) in glass aquaria (60x30x30 cm). Each aquarium was filled with dechlorinated tap water and then stocked with 10 fingerlings (weighing 0.24± 0.02 g and 2.85±0.09 cm in length) procured from “Jyotisar Fish Seed Farm”, Jyotisar, Kurukshetra. Ten experimental isoenergetic and isonitrogenous diets (C, D1-D9) with 40% crude protein were formulated using processed soybean as the major protein source. Different concentrations of phosphorus as di-hydrogen-orthophosphate (0.25, 0.375 and 0.5 $100g^{-1}$ of diet) and calcium as calcium chloride (0.5, 0.75 and 1.0 g 100 g^{-1} of diet) were added to the basal diet. Diet C with no supplementary Ca or P served as the control. Experimental diets were supplemented with additional calcium (Ca) and phosphorus (P) in ratios 0:0 (C), 5.0:2.5 (D1), 5.0:3.75 (D2), 5.0:5.0 (D3), 7.5:2.5 (D4), 7.5:3.75 (D5), 7.5:5.0 (D6), 10:2 (D7), 10:3.75 (D8), 10:5.0 (D9), respectively and fed daily at 4% body weight in two instalments at 8:00 and 16:30 hours for 90 days in triplicates. The amount of feed was adjusted fortnightly following a bulk weighing of each group of fish. The Ca and P content of the holding water were 62.64±2.60 mg l^{-1} and 0.04±0.001 mg l^{-1}, respectively. Dietary ingredient and proximate composition of formulated diets are given in Table 1.

Fish were exposed to the diet for 3 h during each ration; thereafter, the uneaten feed was siphoned out, stored and weighed for calculating feed conversion ratio (FCR). Faecal matter voided by the fish was collected each morning by siphoning approximately 14 h after the removal of the uneaten food. Faeces were oven dried (60ºC) for subsequent analysis. Growth and digestibility were estimated following standard procedure (Garg *et al.,* 2002a).

During experimentation, the water samples were collected from all the experimental aquaria to analyze temperature, dissolved oxygen (DO), pH, electrical conductivity, total alkalinity, total ammonia (NH_4-N) and orthophosphate following APHA (1998) to investigate the influence of compounded feeds on quality of holding water at regular intervals of time.

At the end of 90 days of feeding trials, fingerlings were offered same diet in sufficient quantity, made available for 2h for the feed to be consumed. Then, fixed levels of water were maintained and excess of feed was removed. After that, water samples from each aquaria/tub were collected at two hours intervals to estimate the excretory levels of total ammonia (N-NH_4) and reactive orthophosphate (o-PO_4) following APHA (1998), and were calculated following Sumagaysay-Chavoso (2003).

$$\text{Total ammonia excretion} = \frac{NH_4 - N\,(Mgl^{-1})\text{ in aquarium water}}{\text{Fish weight (Kg) per L of water}}$$

$$\text{Reactive phosphate excretion} = \frac{o - PO^4\,(Mgl^{-1})\text{ in aquarium water}}{\text{Fish weight (Kg) per L of water}}$$

Table 1. Ingredient and proximate composition of experimental diets with different levels of calcium and phosphorus (g Kg^{-1} of diet)

Ingredients	C	D1	D2	D3	D4	D5	D6	D7	D8	D9
Calcium	0	5.0	5.0	5.0	7.5	7.5	7.5	10.0	10.0	10.0
Phosphorus	0	2.5	3.75	5.0	2.5	3.75	5.0	2.5	3.75	5.0
Ingredient composition										
Groundnut cake	650.0	650.0	650.0	650.0	650.0	650.0	650.0	650.0	650.0	650.0
Rice bran	42.0	34.5	33.3	32.0	32.0	30.8	29.5	29.5	28.3	27.0
Processed soybean*	266.0	266.0	266.0	266.0	266.0	266.0	266.0	266.0	266.0	266.0
Wheat flour	32.0	32.0	32.0	32.0	32.0	32.0	32.0	32.0	32.0	32.0
Chromic oxide(Cr_2O_3)	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Calcium**	0	5.0	5.0	5.0	7.5	7.5	7.5	10.0	10.0	10.0
Phosphorus***	0	2.5	3.75	5.0	2.5	3.75	5.0	2.5	3.75	5.0
Mineral mixture****	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Proximate composition										
Crude protein (%)	$39.85^{A}\pm$ 1.36	$39.58^{A}\pm$ 1.15	$40.34^{A}\pm$ 2.10	$39.85^{A}\pm$ 1.36	$39.21^{A}\pm$ 0.79	$40.42^{A}\pm$ 0.49	$40.85^{A}\pm$ 0.80	$39.98^{A}\pm$ 1.16	$41.31^{A}\pm$ 1.43	$39.85^{A}\pm$ 1.36
Crude fat (%)	$9.10^{A}\pm$ 0.26	$8.16^{A}\pm$ ±0.38	$8.19^{A}\pm$ 0.63	$8.58^{A}\pm$ 0.33	$7.78^{B}\pm$ 0.11	$8.44^{A}\pm$ 0.29	$8.78^{A}\pm$ 0.16	$9.65^{A}\pm$ 0.38	$8.56^{A}\pm$ 0.47	$9.10^{A}\pm$ 0.26
Crude fiber (%)	$6.23^{A}\pm$ 0.06	$6.38^{A}\pm$ 0.36	$6.08^{A}\pm$ 0.03	$6.38^{A}\pm$ 0.36	$6.35^{A}\pm$ 0.33	$5.74^{A}\pm$ 0.31	$5.74^{A}\pm$ 0.31	$6.77^{A}\pm$ 0.17	$6.14^{B}\pm$ 0.10	$6.23^{B}\pm$ 0.06
Total ash (%)	$6.62^{A}\pm$ 0.39	$6.49^{A}\pm$ 0.60	$6.45^{A}\pm$ 0.18	$6.19^{A}\pm$ 0.07	$6.71^{A}\pm$ 0.24	$6.51^{A}\pm$ 0.41	$6.57^{A}\pm$ 0.36	$7.51^{A}\pm$ 0.21	$7.45^{A}\pm$ 0.26	$6.60^{B}\pm$ 0.39
Moisture (%)	7.41± 0.20^{A}	7.26± 0.44^{A}	6.66± 0.14^{A}	7.86± 0.11^{A}	7.67± 0.06^{A}	7.26±0. 0.22^{A}	6.89 0.08^{A}	7.68± 0.23^{A}	8.27± 0.21^{A}	7.41± 0.20^{A}
Nitrogen free extract (%)	$29.30^{A}\pm$ 1.42	$30.74^{A}\pm$ 2.70	$30.46^{A}\pm$ .67	$29.95^{A}\pm$ 1.03	$30.80^{A}\pm$ 1.15	$30.46^{A}\pm$ 0.56	$29.62^{A}\pm$ 0.61	$26.58^{A}\pm$ 1.25	$25.65^{A}\pm$ 1.61	$29.30^{A}\pm$ 1.42
Gross energy (kJ g^{-1})	$17.93^{A}\pm$ 0.09	$17.85^{A}\pm$ 0.12	$17.99^{A}\pm$ 0.24	$17.90^{A}\pm$ 0.09	$17.62^{A}\pm$ 0.06	$18.12^{A}\pm$ 0.15	$18.20^{A}\pm$ 0.05	$17.65^{B}\pm$ 0.06	$17.78^{B}\pm$ 0.06	$17.93^{A}\pm$ 0.09
Feed phosphorus (%)	$1.48^{D}\pm$ 0.11	$1.66^{C}\pm$ 0.21	$1.80^{B}\pm$ 0.04	$1.96^{A}\pm$ 0.22	$1.55^{B}\pm$ 0.14	$1.76^{A}\pm$ 0.04	$1.53^{B}\pm$ 0.17	$1.8^{A}\pm$ 0.10	$1.90^{A}\pm$ 0.03	$1.98^{A}\pm$ 0.11

*Soybean was hydrothermically processed in an autoclave at 121° C (15 lbs for 15 minutes) to eliminate antinutrient factors (Garg *et al.*, 2002b); **Calcium chloride ($CaCl_2.2H_2O$) ***Potassium dihydrogen orthophosphate (KH_2PO_4); ****Each kg has nutritional value: copper 312 mg, cobalt 35 mg, magnesium 2.114g, iron 979 mg, zinc 2 mg, iodine 15 mg, DL-methionine 1.920 g, L-lysine monohydrochloride 4.4 g, calcium 30%, phosphorous 8.25%; All values are Mean ± S. E. of mean; Means with different letters in the same row are significantly ($P<0.05$) different (Duncan's Multiple Range test).

At the termination of the experiment, from each treatment, fishes were obtained and kept on an ice tray. Intestine of fish were extirpated and processed for determination of protease (Walter, 1984) and amylase activity (Sawhney and Singh, 2000).

The moisture content, ash, and protein content of the diets and the fish carcasses were analyzed according to AOAC (1995): moisture content after drying in an oven at 104ºC until constant weight; ash content by incineration in a muffle furnace at 600ºC for 24 hours; crude protein by the Kjeldahl method.

Significant differences among treatment groups were tested by Analysis of variance (ANOVA) followed by Duncan's multiple range tests (Duncan, 1955). Statistical significance were settled at a probability value of P<0.05. All statistics were performed using suitable SPSS Version 16.0.

RESULTS AND DISCUSSION

This experiment was designed to provide information on growth responses, survival rate, carcass composition of fingerlings of *C. mrigala* when fed on diets supplemented with nine different concentrations (%) of calcium (Ca) and phosphorus (P). The aim was to determine optimum calcium and phosphorus levels required for the optimum growth of experimental fish. All groups of fingerlings accepted the respective experimental diets satisfactorily and maintained normal behaviour throughout the experimental period.

The growth responses of the *C. mrigala* fed on experimental diets (D1-D9) and control diet are shown in Table 2. Survival was high in all treatments. Growth performance in terms of live weight gain (g) was high in diet D5 where phosphorus was added at 3.75 g kg^{-1} and calcium at 7.5 g kg^{-1}. Growth (%) gain in BW, growth per day (%) in BW, SGR were significantly higher in fingerlings fed on D5 diet (phosphorus was added at 3.75 g kg^{-1} and calcium at 7.5 g kg^{-1}). Further, the growth increased with increase in the dietary P supplementation i.e. from 2.5 g kg^{-1} to 3.75 g kg^{-1}, and thereafter growth declined, similarly growth increased with increase in Ca levels i.e. from 0.5 to 0.75% and thereafter a decline was observed. The deficiency signs observed in this experiment were reduced growth, low digestibility and high feed conversion ratio in diets containing other ratios. In the present studies it was observed that phosphorus should be supplemented at 0.375 g 100g^{-1} (3.75 g kg^{-1}) and Ca should be supplemented between 0.5 and 0.75 g 100g^{-1} (5.0 and 7.5 g kg^{-1} of diet).

Zhang *et al.* (2006) reported that a phosphorus deficiency produced lower bone ash in Japanese seabass, while Ketola (1975) found that supplementation of organic phosphorus in the diet significantly increased bone ash content for Atlantic

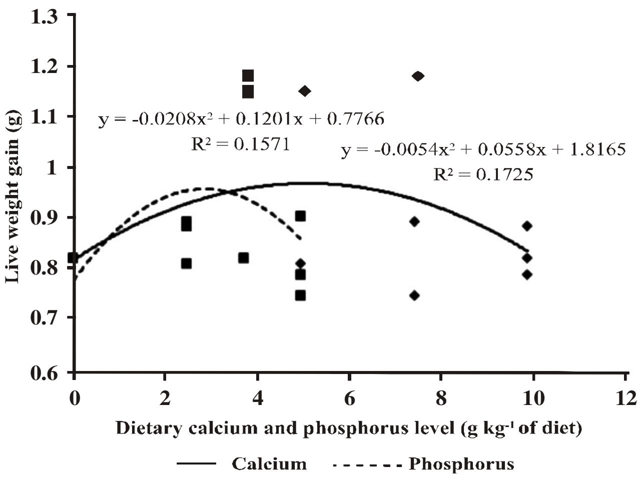

Fig. 1. Polynomial fit curve to show dietary calcium and phosphorus requirement fitting to data of live weight gain

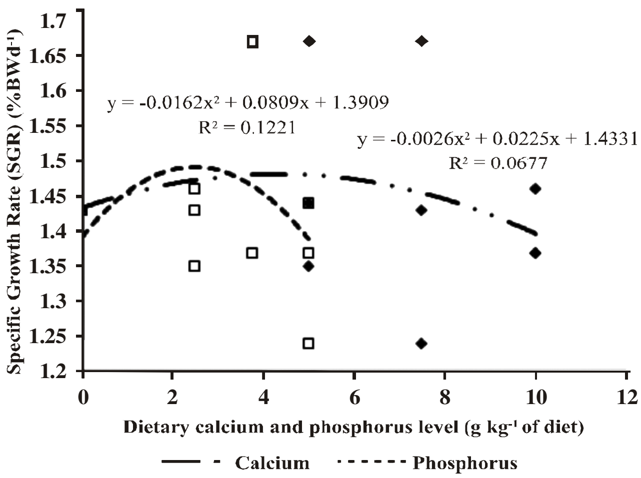

Fig. 2. Polynomial fit curve to show dietary calcium and phosphorus requirement fitting to data of specific growth rate

Table 2. Growth performance and intestinal enzyme activity of *C. mrigala* fingerling fed on different levels of phosphorus and calcium supplementation

Ingredients	C	D1	D2	D3	D4	D5	D6	D7	D8	D9
Calcium	0	5.0	5.0	5.0	7.5	7.5	7.5	10.0	10.0	10.0
Phosphorus	0	2.5	3.75	5.0	2.5	3.75	5.0	2.5	3.75	5.0
Initial weight (g)	$0.31^{A}\pm$ 0.02	$0.34^{A}\pm$ 0.01	$0.32^{A}\pm$ 0.01	$0.31^{A}\pm$ 0.02	$0.33^{A}\pm$ 0.01	$0.32^{A}\pm$ 0.01	$0.34^{A}\pm$ 0.01	$0.32^{A}\pm$ 0.01	$0.33^{A}\pm$ ±0.01	$0.32^{A}\pm$ 0.01
Final weight (g)	$1.13^{C}\pm$ 0.01	$1.15^{B}\pm$ 0.01	$1.48^{A}\pm$ 0.03	$1.24^{B}\pm$ 0.13	$1.22^{B}\pm$ 0.17	$1.51^{A}\pm$ 0.02	$1.09^{C}\pm$ 0.13	$1.20^{A}\pm$ 0.02	$1.16^{B}\pm$ 0.01	$1.12^{B}\pm$ 0.05
Live weight gain (g)	$0.82^{C}\pm$ 0.03	$0.81^{C}\pm$ 0.02	$1.15^{A}\pm$ 0.04	$0.90^{B}\pm$ 0.14	$0.89^{B}\pm$ 0.15	$1.18^{A}\pm$ 0.02	$0.75^{C}\pm$ 0.23	$0.88^{A}\pm$ 0.01	$0.82^{B}\pm$ 0.01	$0.79^{B}\pm$ 0.05
Survival rate (%)	100^{A}	100^{A}	89.96^{A}	90.48^{A}	100^{A}	100^{A}	100^{A}	100^{A}	100^{A}	95.24^{A}
Growth (%) gain in BW	$268.1^{B}\pm$ 14.67	$239.12^{BC}\pm$ 13.78	$355.42^{A}\pm$ 28.97	$264.86^{B}\pm$ 43.45	$267.43^{B}\pm$ 36.97	$362.75^{A}\pm$ 12.56	$223.90^{C}\pm$ 31.34	$273.86^{A}\pm$ 7.70	$245.0^{B}\pm$ 10.31	$246.50^{B}\pm$ 17.51
Growth per day (%) in BW	$1.26^{B}\pm$ 0.06	$1.20^{BC}\pm$ 0.02	$1.41^{A}\pm$ 0.04	$1.26^{B}\pm$ 0.10	$1.25^{B}\pm$ 0.07	$1.42^{A}\pm$ 0.01	$1.11^{C}\pm$ 0.17	$1.27^{A}\pm$ 0.01	$1.21^{B}\pm$ 0.02	$1.19^{B}\pm$ 0.05
Specific growth rate (SGR) (% BW d^{-1})	$1.43^{B}\pm$ 0.10	$1.35^{C}\pm$ 0.04	$1.67^{A}\pm$ 0.06	$1.44^{B}\pm$ 0.15	$1.43^{B}\pm$ 0.11	$1.67^{A}\pm$ 0.04	$1.24^{C}\pm$ 0.17	$1.46^{A}\pm$ 0.02	$1.37^{B}\pm$ 0.03	$1.37^{B}\pm$ 0.05
Feed conversion ratio (FCR)	$1.18^{B}\pm$ 0.04	$1.31^{A}\pm$ 0.03	$1.05^{C}\pm$ 0.04	$1.30^{A}\pm$ 0.09	$1.42^{B}\pm$ 0.27	$1.04^{C}\pm$ 0.01	$1.76^{A}\pm$ 0.25	$1.24^{A}\pm$ 0.02	$1.31^{A}\pm$ 0.02	$1.33^{A}\pm$ 0.08
Gross conversion efficiency (GCE)	$0.84^{B}\pm$ 0.03	$0.75^{BC}\pm$ 0.01	$0.95^{A}\pm$ 0.03	$0.80^{B}\pm$ 0.12	$0.74^{BC}\pm$ 0.13	$0.94^{A}\pm$ 0.01	$0.67^{C}\pm$ 0.21	$0.80^{A}\pm$ 0.01	$0.75^{A}\pm$ 0.01	$0.75^{A}\pm$ 0.05
Protein efficiency ratio (PER)	$0.02^{A}\pm$ 0.01	$0.01^{B}\pm$ 0.01	$0.02^{A}\pm$ 0.01	$0.02^{A}\pm$ 0.01	$0.02^{A}\pm$ 0.01	$0.02^{A}\pm$ 0.00	$0.01^{B}\pm$ 0.01	$0.02^{A}\pm$ 0.00	$0.02^{A}\pm$ 0.01	$0.01^{A}\pm$ 0.01
Apparent protein digestibility (APD %)	$76.8^{C}\pm$ 0.15	$82.5^{B}\pm$ 0.16	$86.3^{A}\pm$ 0.35	$83.2^{B}\pm$ 0.7	$85.1^{B}\pm$ 0.32	$87.2^{A}\pm$ 0.5	$80.1^{BC}\pm$ 0.2	$79.3^{A}\pm$ 0.2	$77.6^{AB}\pm$ 0.3	$72.3^{C}\pm$ 0.04
Specific protease activity[1]	$3.82^{B}\pm$ ±0.17	$3.87^{B}\pm$ 0.32	$4.70^{A}\pm$ 0.29	$4.1^{B}\pm$ 0.27	$4.03^{B}\pm$ 0.08	$5.53^{A}\pm$ 0.52	$3.69^{C}\pm$ 0.45	$4.00^{A}\pm$ 0.06	$3.58^{B}\pm$ 0.09	$3.54^{B}\pm$ 0.33
Specific amylase activity[2]	$3.55^{B}\pm$ 0.24	$3.46^{B}\pm$ 0.09	$4.53^{A}\pm$ 0.21	$4.27^{B}\pm$ 0.23	$4.19^{B}\pm$ .09	$4.73^{A}\pm$ 0.13	$4.16^{B}\pm$ 0.18	$3.69^{A}\pm$ 0.07	$3.49^{B}\pm$ 0.07	$3.44^{B}\pm$ 0.07

[1]mg of tyrosine liberated mg of protein $^{-1}$ h^{-1}; [2]mg of maltose liberated mg of protein $^{-1}$ h^{-1}; All values are Mean±S.E of mean; Means with different letters in the same row are significantly ($P<0.05$) different (Duncan's Multiple Range test).

salmon. The dietary phosphorus levels greatly affected the ash, calcium and phosphorus contents of the bones of chum salmon (Watanbe *et al.,* 1980). Robinson *et al.* (1987) found that 0.50 g 100g^{-1} of phosphorus was adequate for normal bone mineralization based on calcium levels in bone ash. In contrast, Rodehutscord (1996) was unable to confirm any necessity for phosphorus for maximum mineralization of rainbow trout. Rodgers (1984) performed experiments with brook trout using labelled calcium, showed that ambient calcium concentration had a pronounced effect on calcium dynamics. In the present study mineral contents (Ca and P) of bone and scale were not separately monitored, however, carcass ash content and phosphorus appeared to be positively correlated with growth performance. These results correlate well with the dietary calcium and phosphorus contents.

No significant difference was observed in APD of diets. On the other hand, FCR value was significantly lower in fingerlings fed on D5 diet. Growth rate was high in the group of fingerlings fed on diet D5 supplemented with Ca and P at 7.5 g kg^{-1} and 3.75 g kg^{-1} of diet. No significant (P<0.05) variation was observed in GCE and protein efficiency ratio of all diets. Polynomial fit curves denote the relationship of different growth parameters with different levels of calcium and phosphorus (Fig. 1 and 2). Present studies on *C. mrigala* indicate that increasing dietary calcium level (beyond 0.75 g 100g^{-1}) had a negative effect not only on growth and digestibility (APD) but also on other physiological parameters like specific activities of protease and amylase enzymes observed in the present study. Nakamura (1982) also found that increasing dietary calcium had a negative relationship with amount of phosphorus absorbed. On the other hand, Sakamoto and Yone (1973) showed that some calcium was necessary in diets containing high levels of phosphorus because the uptake of the calcium from water was not sufficient to meet the requirements of growth in red sea bream *Crysophrys major*. Maximum growth of catfish was obtained in experiments where diets contained 1.5% calcium and considered by Andrews *et al.* (1973) that the absence of a growth-response relationship at levels below 1.5% calcium indicated a requirement of at least this level in the diet. Robinson *et al.* (1987) found that weight gain and feed conversion rate was optimum for good growth at 0.70% dietary calcium in *O. aureus*, but this did not clearly reflects bone or scale mineralization.

Garg *et al.* (2002b) have further reported that hydrothermal treatment only slightly reduces phytase phosphorus (from 36.14 to 34.2 µg g^{-1}). Hence, the phosphorus must be supplemented into the diet at optimum level, due to the function of P in the bone structure, an increasing supply there of significantly increases the P content of the bone and thus its mineralization (Ogino *et al.,* 1979). However, much of the phosphorus in commercial fish diets may be released into the environment (Wiesmann *et al.,* 1988) and this is influenced both by the availability of dietary phosphorus and the high levels of phosphorus encountered in feeds because of the high levels found in animal proteins such as fishmeal. The gross requirement of P is significantly affected by its availability in the intestines. The relative availability varies greatly with fish species, diet composition and form of P (Schwartz, 1995). Keeping in view the above points when in the present studies diets D1 to D9 supplemented with varying phosphorus levels were fed to the *C. mrigala* fingerlings it was found that optimum phosphorus level is 0.375 per cent. The results of the present investigation were in accordance with the results observed in Japanese seabass (*Lateolavrax japonicus*) (Zhang *et al.,* 2006), black seabream (*Sparus macrocephalus*) (Shao *et al.,* 2008) and Chinese sucker (Yuan *et al.,* 2011).

Intestinal digestive enzyme activity of two enzymes i.e. protease and amylase were determined. Data revealed that specific activity of digestive enzymes was high in diet D5 supplemented with Ca and P at 7.5 g kg^{-1} and 3.75 g kg^{-1} of diet followed by control (Table 2). A definite relationship of dietary Ca-P ratio and the growth performance as also shown by polynomial fit curves (Fig. 1, 2, 3) of this fish species and their nutritive physiology in terms of specific activities of digestive enzymes (protease and amylase) was observed in the present study. Studies by Paul *et al.* (2004) confirmed these results in *C. mrigala*. However,

Table 3. Effect of fish fed with different levels of phosphorus and calcium supplementation on water quality characteristics

Ingredients	C	D1	D2	D3	D4	D5	D6	D7	D8	D9
Calcium	0	5.0	5.0	5.0	7.5	7.5	7.5	10.0	10.0	10.0
Phosphorus	0	2.5	3.75	5.0	2.5	3.75	5.0	2.5	3.75	5.0
Dissolved oxygen	5.16^{A}±	5.00^{A}±	5.05^{A}±	5.09^{A}±	5.90^{A}±	5.78^{A}±	5.89^{A}±	5.07^{A}±	5.95^{A}±	5.94^{A}±
DO) (mg l^{-1})	0.07	0.09	0.08	0.07	0.09	0.07	0.07	0.06	0.07	0.05
pH	8.15^{A}±	8.21^{A}±	8.21^{A}±	8.22^{A}±	8.17^{A}±	8.22^{A}±	8.20^{A}±	7.86^{A}±	8.21^{A}±	8.13^{A}±
	0.01	0.30	0.02	0.01	0.03	0.04	0.01	0.31	0.02	0.02
Conductivity (µ mho cm^{-1})	572.4^{A}±	545.8^{A}±	548.04^{A}±	533.3^{A}±	567.84^{A}±	579.1^{A}±	520.19^{B}±	566.32^{A}±	566.32^{A}±	576.6^{A}±
	13.10	13.43	16.05	15.55	9.59	23.34	26.78	13.84	13.84	23.75
Carbonates (mg l^{-1})	14.77^{B}±	14.21^{B}±	17.75^{A}±	20.42^{A}±	18.21^{A}±	18.64^{A}±	18.66^{A}±	19.55^{A}±	19.54^{A}±	18.22^{A}±
	6.95	2.48	1.62	4.97	2.48	4.01	2.66	4.64	3.88	2.22
Bicarbonates (mg l^{-1})	128.87^{A}±	163.93^{A}±	143.53^{A}±	135.98^{A}±	138.64^{AB}±	140.88^{A}±	145.31^{A}±	146.18^{A}±	137.73^{AB}±	131.54^{AB}±
	30.05	12.73	34.52	38.71	34.73	37.17	34.29	27.86	43.05	28.28
Chloride (mg l^{-1})	31.77^{A}±	33.31^{A}±	31.98^{A}±	32.41^{A}±	33.97^{A}±	33.75^{A}±	34.19^{A}±	33.52^{A}±	31.54^{A}±	31.77^{A}±
	2.49	1.87	1.14	2.30	2.15	2.49	2.08	2.52	2.28	2.49
Total Ammonia excretion	1647.76^{A}±	1163.03^{B}±	708.33^{D}±	1036.73^{C}±	812.26^{C}±	607.16^{D}±	1213.80^{B}±	1408.46^{D}±	1747.20^{B}±	1810.10^{A}±
(mg kg^{-1} BW day^{-1})	19.58	22.19	22.20	24.57	18.73	19.82	22.57	19.11	32.24	16.0
Total phosphate production	756.73^{A}±	514.43^{B}±	320.06^{C}±	458.03^{BC}±	434.93^{B}±	258.73^{C}±	465.83^{B}±	816.5^{B}±	816.5^{B}±	824.5^{A}±
(mg kg^{-1} BW day^{-1})	21.96	17.41	19.08	19.96	19.96	20.60	21.00	23.19	23.19	29.59

All values are Mean±S.E of mean; Means with different letters in the same row are significantly ($P<0.05$) different; (Duncan's Multiple Range test);
[1]mg of tyrosine liberated mg of protein $^{-1}$ h^{-1}; [2]mg of maltose liberated mg of protein $^{-1}$ h^{-1}; All values are Mean±S.E of mean; Means with different letters in the same row are significantly ($P<0.05$) different (Duncan's Multiple Range test).

Table 4. Proximate composition of fish carcass fed on different levels of phosphorus and calcium supplementation

Proximate	Initial value	C	D1	D2	D3	D4	D5	D6	D7	D8	D9
Moisture (%)	$71.27^{A}\pm$ 0.05	$68.22^{A}\pm$ 0.02	$67.85^{A}\pm$ 0.02	$67.29^{A}\pm$ 0.05	$67.71^{A}\pm$ 0.03	$67.62^{A}\pm$ 0.02	$67.35^{A}\pm$ 0.02	$67.72^{A}\pm$ 0.03	$67.52^{A}\pm$ 0.51	$67.19^{A}\pm$ 0.01	$68.07^{A}\pm$ 0.01
Crude protein (%)	$8.08^{D}\pm$ 0.04	$12.33^{A}\pm$ 0.17	$12.07^{B}\pm$ 0.03	$15.10^{A}\pm$ 0.15	$10.08^{C}\pm$ 0.14	$12.69^{B}\pm$ 0.24	$16.63^{A}\pm$ 0.08	$9.81^{C}\pm$ 0.11	$12.58^{A}\pm$ 0.14	$9.47^{B}\pm$ 0.14	$8.16^{B}\pm$ 0.20
Crude fat (%)	$2.83^{C}\pm$ 0.05	$6.83^{A}\pm$ 0.04	$3.80^{B}\pm$ 0.03	$5.00^{AB}\pm$ 0.15	$3.75^{B}\pm$ 0.06	$3.78^{B}\pm$ 0.02	$5.08^{A}\pm$ 0.05	$3.63^{B}\pm$ 0.06	$3.75^{B}\pm$ 0.07	$3.62^{B}\pm$ 0.05	$3.58^{B}\pm$ 0.02
Total ash (%)	$3.52^{C}\pm$ 0.01	$3.91^{B}\pm$ 0.03	$4.03^{A}\pm$ 0.02	$4.33^{A}\pm$ 0.03	$4.28^{A}\pm$ 0.01	$4.30^{B}\pm$ 0.02	$4.58^{A}\pm$ 0.03	$4.15^{C}\pm$ 0.02	$4.30^{A}\pm$ 0.03	$4.11^{A}\pm$ 0.03	$3.97^{B}\pm$ 0.02
Nitrogen free extract (%)	$14.28^{A}\pm$ 0.11	$8.7^{C}\pm$ 0.19	$12.23^{B}\pm$ 0.09	$8.28^{C}\pm$ 0.15	$14.17^{A}\pm$ 0.04	$11.60^{B}\pm$ 0.31	$6.34^{C}\pm$ 0.14	$14.67^{A}\pm$ 0.10	$11.84^{B}\pm$ 0.20	$15.61^{A}\pm$ 0.16	$16.20^{A}\pm$ 0.17
Gross energy (kJ g^{-1})	$5.46^{C}\pm$ 0.003	$7.10^{A}\pm$ 0.02	$6.43^{B}\pm$ 0.01	$6.96^{A}\pm$ 0.04	$6.28^{B}\pm$ 0.01	$6.48^{B}\pm$ 0.01	$7.02^{A}\pm$ 0.01	$6.27^{B}\pm$ 0.01	$6.48^{A}\pm$ 0.02	$6.23^{B}\pm$ 0.13	$6.11^{B}\pm$ 0.02

All values are Mean±S.E of mean; Means with different letters in the same row are significantly ($P<0.05$) different; (Duncan's Multiple Range test)

according to which diets having 1.9 kg^{-1} Ca and 7.5 kg^{-1} P obtained best growth.

The data on water quality characteristics pertaining to all the dietary treatments is presented in Table 4. No significant variations were observed in water quality parameters. In general, significantly ($P<0.05$) low values in total ammonia excretion and reactive phosphate production (mg kg^{-1} BW d^{-1}) were recorded in fish fed on diet D5 supplemented with Ca and P at 7.5 g and 3.75 g kg^{-1} of diet (Fig. 3). The excretion of wastes into the effluent water remained significantly ($P<0.05$) low when fish were fed on diet supplemented with Ca/P ratio between the range 1.33 to 2.00. Peak values in total ammonia excretion occurred at 6 h post feeding, whereas the level of soluble phosphate in the aquaria water was high initially, at 2 h post feeding and later on values declined. These results are in broad agreement to those of Kalla and Garg (2003) and Jana *et al.* (2006). The excretion patterns observed in the present trial, however, differ from those obtained by Sumagaysay-Chavoso (2003) in milkfish fed on formulated and natural food-based diets in that the excretory levels in his studies were very low and obtained two peaks (6 h and 18 h) post feeding for total ammonia nitrogen (TAN) and only one peak, at 21 h post feeding, for o-PO_4.

Phosphorus metabolism is closely tied to that of calcium. Any excess minerals in the diet above the

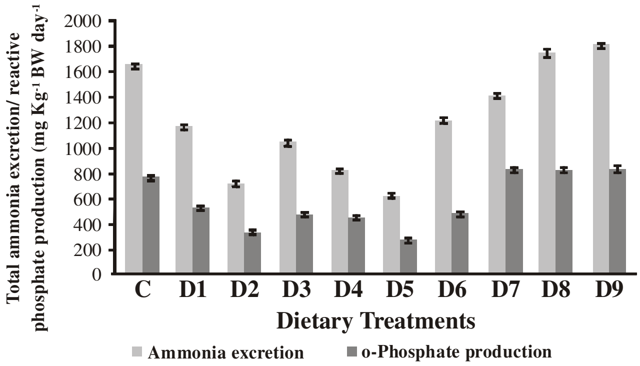

Fig. 3. Post prandial excretory patterns of total ammonia and total orthophosphate (mg kg^{-1} Body weight per day of fish) in holding water for fish *C. mrigala* in different dietary treatments containing varying levels of calcium and phosphorus

optimum requirement for fish will be excreted by the fish in the water. It is, therefore, critical to know precisely the dietary requirement of P and Ca in order to minimize excess P in diet without risking P deficiency in cultured fish. It has been further confirmed that ability of *C. mrigala* to benefit from additional calcium and P in the diet in the ratio between 1.33 (Ca 5.0:P 3.75) – 2.0 (Ca 7.5:P 3.75) for optimum growth and efficient feed utilization.

The carcass composition with respect to proximate nutrients of *C. mrigala* on the basis of feeding trial is shown in Table 4. The accumulation of carcass crude protein (%) was significantly ($P<0.05$) higher in the carcass of fishes fed on diet D5. Crude fat was significantly higher in control group. No significant variation ($P<0.05$) were observed in moisture (%) and total ash content (%) of fishes fed on different diets, whereas, NFE (%) was significantly ($P<0.05$) high in D9 fed group as compared to control. However, there were no significant ($P<0.05$) variations in gross energy (kJ g^{-1}) in the experimental diets fed group.

The results showed that significantly ($P<0.05$) higher growth performance in terms of weight gain, SGR, growth % gain in BW was observed in the group of fishes fed on diets supplemented with Ca and P at 7.5 and 3.75 g kg^{-1} of diet (D5). FCR values were also low in D5. Results of present studies have further revealed that fish fed on diets supplemented with Ca-P ratio in the range of 1.33-2.0 had significantly ($P<0.05$) higher carcass protein, fat, ash and energy and a lower percentage of moisture in comparison with fish fed on other diets. Guerreiro *et al.* (2004) reported whole-body Ca uptake in gilthead sea bream (*Sparus aurata*) larvae during short-term adaptation to altered salinities improved when the Ca concentration in the rearing water was increased. Our studies are at par with other similar studies (Paul *et al.,* 2004) in *C. mrigala* fingerlings. Chavez-Sanchez *et al.* (2000) and Wang *et al.* (2003) showed strong positive correlation between calcium and phosphorus in the diet and ash content in the carcass of the fish, demonstrating the role of these components in mineralization. Similar results were observed by Sugiura *et al.* (2004) in several fish species.

CONCLUSIONS

Supplementation of 3.75 g kg^{-1} of phosphorus and 7.5 g kg^{-1} of calcium was found to be optimum for improved growth performance of *Cirrhinus mrigala* fingerlings.

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