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Further Studies on Protein Requirements of Growing Indian Major Carps Under Field Conditions

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Abstract

The purpose of this study was to determine optimum protein levels required for Indian major carp fry (mean body weight 1.95 g). Fry were stocked @ 10 million ha^{-1} in nursery ponds and fed on one of the five formulated diets (protein content ranged between 33-45%) over a period of 40 days. Growth and specific growth rate (SGR) values remained low when fed on low dietary protein levels (Treatments 1 and 2). Highest growth performance (weight gain, SGR and length) was observed in nursery ponds where the fry were fed on 40% dietary protein irrespective of the protein source (soycake or fish meal, Treatments 3 and 4). An increase in the dietary protein levels (beyond 40%) not only repressed growth performance but also affected survival rate. An investigation on the effects of feeds on water quality parameters revealed that nutrients ($o\text{–}PO_4$, Kjeldahl nitrogen, $NO_3\text{–}N$) and all the productivity indicating parameters viz. chlorophyll ‘a’, net primary productivity (NPP), and plankton population, were high in treatments 3 and 4, where the fish were fed on 40% dietary protein. In general, $N\text{-}NH_4$ levels remained high in ponds where the fry were fed on diets containing fish meal as the protein source irrespective of the protein levels. Multivariate analysis of the data also revealed a significant ($P < 0.05$) positive correlation of nutrients, chlorophyll ‘a’, NPP, GPP and plankton population with fry growth. The present study indicated that to obtain high growth rates (under field conditions) farmers may have to feed the fry/advanced fry on supplementary diets containing about 40% protein preferrably of plant origin.

Introduction

Indian major carp is one of the most important group of fishes cultured in the Indian subcontinent and accounts for more than 95% of the

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world production. Pond fertilization with organic and inorganic fertilizers are effective methods for increasing primary productivity. However, their excessive use deteriorates the water quality (Boyd 1992). These fishes thrive low in the food web and feed on some of the farm yard wastes, which do not meet their nutritional requirements. Further, high stocking rates also lead to a reduction of standing crop of natural fish food organisms to such an extent that these organisms do not develop in sufficient number even in case of high productivity. Therefore, for high survival and growth, the natural feed has to be supplemented with balanced supplementary compounded diets.

Fish meal has traditionally been used as a major protein source because of its high protein content and palatability. However, the high cost and short supply of quality fish meal has made it necessary to substitute it with other cheaper protein sources. Since the quality of fish meal available in the interior parts of India is generally very poor, adulteration and microbial infestation are very common. Further, fish meal contains very high percentage of phosphorus. Therefore, alternative dietary protein sources need to be researched (Kikuchi 1999). Even though proteins of plant origin offer great potentials, presence of many endogenous antinutritional factors (Tacon 1993) and toxic substances interfere with appetite, absorption efficiency and metabolism. Therefore, to remove antinutrient factors (ANFs), plant proteins have to be suitably processed before incorporation in the diets, heat treatment and soaking over night before feeding also eliminates some of the ANFs present in the ingredients (Garg et al. 2002; Singh et al. 2003). Furthermore, the enzyme system in cyprinids, which have a long gut, is better equipped to digest and absorb nutrients from plant feedstuffs (Jafri and Anwar 1995). Singh (1992) has also reported high digestibility (86-92%) for plant protein in the yearlings of *C. mrigala* and grass carp. Law (1984) and Hanley (1987) have also reported a higher digestibility coefficient for soybean than for fish meal in grass carp and omnivorous tilapia respectively. Allan and Rowland (1994) and Singh et al. (2003) have also reported high apparent digestibility coefficients for a number of plant products in omnivorous silver perch and *Cirrhinus mrigala*, respectively.

Our earlier studies have revealed that diets consisting of only rice bran and oil-cake commonly used by farmers neither contain essential nutrients in sufficient quantity nor optimum protein levels (Singh 2001). These studies have also revealed that growth and survival appeared to be a function of dietary protein level and the fry continued to grow with increase in dietary protein level, indicating high dietary protein requirements of Indian major carp even under field conditions. Since in these studies highest dietary protein levels were only 35%, therefore, to determine the optimum protein requirements of Indian major carp advance fry, diets containing 33 to 45% crude protein were formulated and fed to the fry stocked in fertilized nursery ponds. In the present studies, therefore, efforts were also made to compare the efficacy of fish meal versus soybean cake containing diets at 40 per cent dietary protein level.

Materials and Methods

Experimental feed and feeding schedule

Five different feeds (1–5) were formulated (Table 1). Ingredients were thoroughly ground and mixed. Soycake was heat processed before incorporation and diets were soaked overnight in water before feeding the same to fry daily in the morning (between 0800 and 0900) @ 5% BWd^{-1}. Three hundred ry from each treatment were bulk weighed at weekly intervals to adjust the feeding rates and to calculate feed conversion ratio (FCR).

Experimental design

The experiment was carried out in earthern ponds (area 0.075 to 0.30 ha, mean water depth 1m) at the fish farm facility of M/s. Royal fish farm, Hisar (Lat. 29°, 10′ N; Long 75°, 46′ E), for a period of 40 days from October to December 2002. Prior to the commencement of treatments, ponds were cleaned, lime was applied (@ 200 $kg \cdot ha^{-1}$) and filled with tubewell water (salinity 0.45 to 0.55 ppt, hardness 1268 to 1300 mg l^{-1}). Water levels were maintained (1 m depth) by replenishing the water daily from the tubewell. Pond fertilization, supplementary feeding and stocking density

Table 1. Ingredient content (%) and proximate composition (% dry weight) of experimental diets (1-5)

	Diets				
	1	2	3	4	5
Ingredients*					
Mustard oilcake	60.0	60.0	-	-	-
Groundnut oilcake	-	-	65.0	65.0	65.0
Rice bran	29.0	19.0	12.75	12.65	2.65
Fish meal	10.0	-	21.25	-	-
Soybean cake[a]	-	20.0	-	21.35	31.35
Mineral premix and amino acids[b] (MPA)	1.0	1.0	1.0	1.0	1.0
Proximate analysis (%)					
Dry matter	91.5	91.8	93.7	92.3	92.4
Crude protein	33.0	35.0	40.2	40.1	45.0
Crude fat	9.5	8.4	6.6	6.2	5.6
Crude fibre	9.2	9.3	7.8	8.5	8.3
Ash	9.1	9.3	11.3	6.7	6.5
Nitrogen free extract	30.7	29.8	27.8	30.8	27.0
Gross energy kJg^{-1}	16.8	16.7	16.9	17.4	17.4

[a]Pre-roasted soycake
[b]Each kg contains: Copper 312 mg, Cobalt 45 mg, Magnesium 2.114g, Iron 979 mg, Zinc 2.130 g, Iodine 156 mg, DL-Methionine 1.920 g, L-Lysine Mono Hydrochloride 4.4 g, Calcium 30%, Phosphorus 8.25%
*Obtained from local market

Table 2. Effect of supplementary diets (1-5) on survival and weight gain in Indian major carp advanced fry under field conditions (duration 40 days)

Dietary treatments		Initial fish stock		Final fish stock				Weight increase			
	Species stocked	Initial mean weight (g)	Initial mean length (cm)	Survival (%)	Final mean weight (g)	Final mean length (cm)	Final mean biomass (g/m^3)	Live weight gain (g)	Percent weight gain	SGR % $g\ d^{-1}$	FCR*
1	Catla	3.2±0.20	5.9±0.07	93.9	6.3±0.14	7.7±0.04	38.1	3.2^{BC} ±0.07	101.6^{BC} ±8.2	1.8^{B} ±0.10	2.8^{B}±0.10
	Rohu	1.5±0.03	4.9±0.03		2.9±0.20	6.4±0.02		1.5^{DC} ±0.10	100.0^{BC} ±6.9	1.7^{BC} ±0.09	
	Mrigal	1.2±0.07	3.9±0.02		2.1±0.04	6.0±0.04		1.0^{D}±0.03	82.14^{DC} ±7.5	1.5^{D} ±0.10	
2	Catla	3.0±0.15	5.8±0.02	93.3	6.6±0.17	7.9±0.05	40.5	3.6^{B} ±0.10	119.2^{B} ±6.6	2.0^{B} ±0.08	2.4^{B}±0.13
	Rohu	1.4±0.10	4.2±0.10		3.1±0.08	6.6±0.04		1.7^{BC} ±0.13	124.5^{AB} ±15.1	2.0^{AB} ±0.17	
	Mrigal	1.2±0.06	3.8±0.01		2.4±0.06	6.1±0.02		1.3^{C} ±0.02	112.2^{C} ±6.0	1.9^{C} ±0.07	
3	Catla	2.9±0.18	6.1±0.02	95.1	7.7±0.10	8.8±0.09	47.5	4.8^{A} ±0.27	168.3^{A} ±18.4	2.5^{A} ±0.18	1.9^{C}±0.04
	Rohu	1.4±0.04	4.7±0.08		3.5±0.16	6.7±0.02		2.0^{AB} ±0.19	144.3^{A} ±16.7	2.2^{A} ±0.17	
	Mrigal	1.2±0.07	4.0±0.19		3.1±0.03	6.5±0.05		1.8^{B} ±0.04	152.2^{B} ±12.5	2.3^{B} ±0.13	
4	Catla	3.0±0.16	6.1±0.03	92.1	8.3±0.47	9.0±0.06	50.4	5.4^{A} ±0.37	179.8^{A} ±11.9	2.6^{A} ±0.10	1.8^{C}±0.08
	Rohu	1.4±0.06	5.1±0.01		3.5±0.13	6.8±0.03		2.1^{A} ±0.09	156.5^{A} ±7.3	2.4^{A} ±0.07	
	Mrigal	1.2±0.06	3.7±0.02		3.5±0.19	6.6±0.03		2.4^{A} ±0.19	202.9^{A} ±21.4	2.8^{A} ±0.17	
5	Catla	3.8±0.93	6.0±0.40	87.4	6.5±0.15	7.7±0.02	36.5	2.7^{C} ±0.21	70.6^{C} ±7.0	1.3^{C} ±0.10	4.3^{A}±0.59
	Rohu	1.7±0.04	4.8±0.05		3.0±0.06	6.5±0.02		1.3^{D} ±0.04	74.9^{C} ±3.5	1.4^{C} ±0.05	
	Mrigal	1.4±0.07	3.7±0.02		2.3±0.12	6.0±0.04		0.9^{D} ±0.06	63.9^{D} ±3.4	1.2^{D} ±0.05	

All ponds were fertilized at biweekly intervals using cow dung @ 10,000 kg $hy^{-1}y^{-1}$ and poultry excreta @ 3000 kg hectare y^{-1}. Rate of feeding @ 5% BWd^{-1}
All values are mean±SE of mean. Means with the same letter/s in same column are not significantly (P>0.05) different.
SGR (%g d^{-1}) = Specific growth rate = [(In Wtf-In Wti)×100]/t
% weight gain = [(Wtf-Wti)/Wti]×100, where, Wti and Wtf denote initial and final weights of fish respectively and t represents time (days).
*FCR was calculated on the basis of total feed given in each treatment

(10 m^2) were the various management practices adopted in these studies (see table 2 for rate and frequency of fertilization).

Stocking

Two replicates per treatment were maintained and two weeks after the application of organic fertilizers, 28 days old Indian major carp advance fry viz. *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* were stocked in the ratio of 4:3:3 @ 10 million•ha^{-1} in October (See table 2 for mean weight and length of the fry).

Ingredients and diets were analyzed for per cent moisture (drying at 105°C overnight), protein (total kJeldahl nitrogen, multiplied by 6.25), fat (Soxhlet method), ash (incineration at 550°C in muffle furnace), crude fiber (alternate acid and alkali washing method) and total phosphorus (acid mixture digestion method) following AOAC (1995). Analysis revealed that protein contents of the diets varied from 33.0–45% (Table 1).

The energy contents of the diets were calculated using the average caloric conversion factors of 0.3954, 0.1715 and 0.2364•kJg^{-1} for lipid, carbohydrate and protein respectively (Henken et al. 1986).

Water quality monitoring

Water samples for the determination of water quality parameters were obtained in replicates of two before sunrise from each treatment at 20 days interval. Water temperature (°C) was recorded daily using digital thermometer. Dissolved oxygen (DO), pH, conductivity and salinity were measured using multiline F-set 3 (E. Merck Ltd. Germany). Turbidity was measured using Nephalo turbidity meter (Systronics) in NTU. All other parameters were determined following APHA (1998).

Biotic communities

For qualitative and quantitative estimations of phyto- and zooplankton, water samples were collected in duplicates from each treatment at 20 days interval. Plankton samples were collected by passing 10 L of water taken from five different locations of each treatment through a plankton net (mesh size 125 µm). The concentrated samples were then carefully transferred to a measuring cylinder and made up to a volume of 40 ml with distilled water. Samples were preserved in small plastic bottles with 5% buffered formalin. Plankton densities were estimated using a Sedgewick Rafter counting cell (Wetzel and Likens 1979) under a binocular microscope.

Net primary productivity (NPP) was determined on the sampling days following the light and dark bottle technique (APHA 1998). Water samples for chlorophyll ‘a’ (mg•l^{-1}) determination were also collected at 20 days interval. A known amount of water was filtered through Whatman filter paper (No. 40) and extracted using acetone (APHA 1998).

Statistical analysis

Data were subjected to ANOVA and multivariate analysis following Prien et al. (1993). Duncan's multiple range test (Duncan 1955) was also applied to find out the significant differences in fish growth, if any between different treatments.

Results

Survival in different treatments was high and varied between 87.4 to 95.1% (Table 2). Fry in different treatments were fed on one of the five formulated diets containing protein levels ranging from 33.0 to 45.0%. ANOVA revealed that irrespective of the species stocked, a significant ($P < 0.05$) high live weight gain, per cent weight gain and SGR were observed in fry fed on diets containing 40% dietary protein, irrespective of the protein source. Fish biomass also remained high in these two treatments (3 and 4). On the other hand, FCR values at 40% dietary protein level remained significantly ($P < 0.05$) low in comparison to other dietary treatments. A review of data further indicates that an increase in dietary protein contents (beyond 40%) not only repressed growth performance but also significantly increased FCR values.

A comparison of two dietary protein sources (viz. fish meal/processed soycake, diet Nos. 3 and 4) indicates no apparent significant differences in the growth performance of catla and rohu fingerlings, however, weight gain in mrigal fry remained significantly high when fed on diet No. 4 ($P < 0.05$) containing processed soycake as the major protein source.

Hydrobiological characteristics

Dissolved oxygen decreased, while pH, total alkalinity, turbidity and total kjeldahl nitrogen increased with each increase in the dietary protein level, however, no significant differences were observed among different treatments (Table 3). BOD was not high in any of the treatment. The NH_4–N and o-PO_4 content were high in the treatments where the fish were fed on fish meal based diets or in the treatment, where the fish were fed on high dietary protein level. Chrlorophyll contents were significantly ($P < 0.05$) low in ponds where the fish were fed at low dietary (35 and 38%) proteins levels, threafter no significant variations were observed. The No_3–N levels were high in ponds, where the fish were fed on 40% dietary protein irrespective of the source, while plankton population (phytoplankton and zooplankton no's l^{-1}) was significantly ($P < 0.05$) high in ponds where the fish were fed on soycake containing diet (40% protein), and their highest values coincided with the highest fish growth. On the other hand, NPP decreased and GPP increased in ponds where the fry were fed either on fish meal based diet (at 40%) or at highest dietary protein level. No significant variations in NO_2-N levels were observed in different treatments.

Discussion

A significant ($P < 0.05$) decrease in FCR, and increase in survival and growth in terms of weight gain, SGR and percent weight gain was observed in ponds, where the fry were fed on a diet containing 40% protein, irrespective of the protein source. A slight high growth performance at 40% dietary protein from processed soycake may be attributed to the high digestibility of plant protein. Low growth and survival of fry at other dietary treatments (treatments 1, 2 and 5) may indicate that the optimum protein requirements of Indian major carps fry is about 40%. Feeding the fry on high dietary protein level (42%), not only repressed growth performance, but also deteriorated the water quality as is evident from low DO and low NPP levels. Recent studies in this laboratory (Kalla and Garg 2004) also revealed that the optimum protein requirements of *C. mrigala* fingerlings are about 40%, when processed full fat soybean or fish meal was used as the protein source in the supplementary diets and any further increase in protein levels repressed growth performance and nutrient retention etc. Singh et al. (1987) had reported that the fry of *C. mrigala* require about 45% protein under laboratory conditions, and any further increase in the protein levels resulted a reduction in growth. Inspite of the fact that ponds were rich with planktonic flora and fauna, SGR and per cent weight gain also appeared to be a function of supplementary diets.

Table 3. Effect of supplementary diets (1-5) on physico-chemical and biological characteristics of nursery ponds

Parameters	Dietary treatments				
	1	2	3	4	5
Dissolved oxygen mg l^{-1}	$5.3^{A}\pm0.10$	$5.1^{AB}\pm0.10$	$4.9^{B}\pm0.10$	$4.9^{B}\pm0.18$	$4.4^{C}\pm0.07$
BOD_5 mg l^{-1}	$3.8^{AB}\pm0.24$	$3.9^{AB}\pm0.20$	$4.4^{A}\pm0.12$	$3.7^{B}\pm0.08$	$3.8^{AB}\pm0.26$
pH	$7.7^{A}\pm0.10$	$7.8^{A}\pm0.10$	$7.8^{A}\pm0.10$	$8.0^{A}\pm0.11$	$8.0^{A}\pm0.19$
Total alkalinity mg l^{-1}	$184.5^{A}\pm6.0$	$184.8^{A}\pm6.40$	$192.8^{A}\pm4.9$	$192.8^{A}\pm4.9$	$195.8^{A}\pm2.1$
Turbidity NTU	$86.0^{A}\pm5.1$	$91.0^{A}\pm4.8$	$95.9^{A}\pm5.5$	$99.3^{A}\pm4.2$	$100.8^{A}\pm4.1$
o-PO_4 mg l^{-1}	$0.2^{B}\pm0.03$	$0.3^{B}\pm0.04$	$0.40^{A}\pm0.05$	$0.3^{AB}\pm0.03$	$0.4^{AB}\pm0.04$
Total kjeldahl nitrogen mg l^{-1}	$7.4^{A}\pm0.17$	$7.7^{A}\pm0.20$	$8.9^{A}\pm0.24$	$9.4^{A}\pm0.27$	$8.7^{A}\pm0.35$
NO_3-N mg l^{-1}	$5.8^{B}\pm0.23$	$6.3^{B}\pm0.23$	$7.1^{A}\pm0.23$	$7.2^{A}\pm0.22$	$6.4^{B}\pm0.17$
NO_2-N mg l^{-1}	$0.3^{A}\pm0.01$	$0.3^{A}\pm0.01$	$0.3^{A}\pm0.01$	$0.3^{A}\pm0.01$	$0.3^{A}\pm0.01$
NH_4-N mg l^{-1}	$0.6^{B}\pm0.02$	0.6 ± 0.02	$0.7^{A}\pm0.04$	$0.6^{B}\pm0.03$	$0.7^{A}\pm0.03$
NPP mg l^{-1}	$1.1^{A}\pm0.10$	$1.3^{A}\pm0.13$	$1.2^{A}\pm0.04$	$1.1^{A}\pm0.09$	$0.7^{B}\pm0.18$
GPP mg l^{-1}	$2.5^{A}\pm0.10$	$2.5^{A}\pm0.10$	$2.6^{A}\pm0.11$	$2.7^{A}\pm0.11$	$2.1^{B}\pm0.19$
Chlorophyll ‘a’ mg l^{-1}	$0.6^{D}\pm0.03$	$0.8^{C}\pm0.03$	$0.9^{AB}\pm0.04$	$1.0^{A}\pm0.03$	$0.9^{AB}\pm0.06$
Phytoplankton No’s l^{-1}	$7625^{B}\pm224$	$8968^{B}\pm1166$	$9562^{B}\pm1058$	$13875^{A}\pm824$	$9343^{B}\pm459$
Zooplankton no’s l^{-1}	$4343^{B}\pm122$	$6281^{AB}\pm742$	$7281^{A}\pm995$	$7500^{A}\pm687$	$6250^{AB}\pm616$

All values are mean±SE of mean of eight replicates. Temperature during the experimental period decreased from 26.9 to 21.9°C.
Means with the same letter/s in the same row are not significantly ($P>0.05$) different.

The effects of five different diets are also very clearly reflected on the physico-chemical and biological characteristics of pond waters. Although DO levels, remained at optimal levels, the values decreased with each increase in dietary protein level. Low DO values in ponds where the fish were fed on 40% dietary protein (soybean cake containing diet) clearly indicate its utilization by the growing fish. Its negative correlation with chlorophyll 'a' (r=–0.31) and nutrients (total Kjeldahl nitrogen r=-0.36, NO_3-N r=-0.37 and o-PO_4, r=-0.44) further confirms the results. On the other hand, DO levels showed a significant negative correlation with NH_4-N (r=-0.32). Though the values were not statistically significant, fish weight gain showed negative correlation, indicating that high NH_4-N suppressed growth.

Nutrients and productivity indicating parameters, viz., total Kjeldahl nitrogen, available nitrogen (NO_3-N), chlorophyll 'a' and plankton population (phytoplankton and zooplankton population) remained significantly ($P < 0.05$) high in ponds where the fry were fed on 40% dietary protein from soybean cake. These values also coincide with high weight gain parameters. Statistically, fish weight gain and increase in fish biomass showed a significant positive correlation with NO_3-N (r=0.33, 0.49), GPP (r=0.36, 0.36), and chlorophyll 'a' (r=0.35, 0.42). Fish biomass gain also showed a significant positive correlation with phytoplankton (r=0.47) and zooplankton (r=0.33) population, clearly revealing that fish growth is also positively correlated with the trophic status of the ponds. Liang et al. (1981), Olah et al. (1986), Teichert–Coddington (1986), Knud-Hansen and Batterson (1994) and Garg and Bhatnagar (1996 1999 and 2000) have also shown significant positive correlation of fish growth with productivity indicating parameters.

Although GPP was significantly ($P < 0.05$) higher in ponds where highest growth was observed, NPP remained low. Low NPP in these ponds may be attributed to the high respiratory rate of biotic population as is also evident from the high phytoplankton and zooplankton population in these ponds (Delince 1992).

Present studies have thus revealed that with the incroporation of pre-roasted cake in the diet (Diet No.4), fry survival and growth improved, whereas, ammonia pollution decreased. Reduction in N-NH_4 levels with the use of processed soybean in diets have also been reported by many other workers (Singh 2001, Singh et al. 2003). These results also indicate that compounded diets with 40% protein contents are required for high survival and growth even in well manured and productive ponds.

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