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Note

Effect of supplementary feeds with different protein levels on growth and production of Indian butter catfish *Ompok bimaculatus* (Bloch, 1794) in fertilised ponds

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ABSTRACT

The study evaluated the effect of supplementary feeds with varying protein levels on the growth and production of *Ompok bimaculatus* (Bloch, 1794) in earthen ponds. The stocking density was 4000 fingerlings ha^{-1} and culture period was 6 months. Feed-1 comprised rice bran and mustard oil cake at 1:1 ratio, whereas, Feed-2 and Feed-3 additionally contained fish meal, wheat flour and vitamin-mineral premix. The crude protein content was estimated as 18.84% in Feed-1, 30.47% in Feed-2 and 35.24% in Feed-3. The water quality parameters and plankton composition were assessed monthly and soil quality was assessed at the beginning and end of the culture period. All parameters were within the ideal limits for pond farming. Fish growth parameters were assessed monthly. The final weight ($p \leq 0.05$) and survival ($p \geq 0.05$) with Feed-2 and Feed-3 were higher than that obtained with Feed-1. Feed conversion ratio (FCR) for Feed-2 and Feed-3 were better as compared to Feed-1. Fish production was 10% more with Feed-2 and 13% more with Feed-3. Benefit costcratio (BCR) was highest with Feed-2. Carcass composition among the treatments showed trivial variation ($p \geq 0.05$). From the results of the study, it can be concluded that 30% dietary protein is ideal for sustainable farming of *O. bimaculatus* in fertilised pond systems.

Keywords: Growth, *Ompok bimaculatus,* Pabda, Pond farming, Protein content, Survival

The silurid fish *Ompok bimaculatus* (Bloch, 1794) is a high-valued catfish in India due to its unique taste and competitive price (Sridhar *et al.,* 1998). This fish is native to the freshwater habitats of India, Pakistan, Sri Lanka, Bangladesh and Myanmar (Ng and Hadiaty, 2009; Kottelat, 2013). It is popularly known as '*pabda*' or 'Indian butter catfish'. In international ornamental fish trade, it is commonly referred as 'two-spot glassy catfish'. It naturally breeds in streams, rivers and wetlands during monsoon, but, wanton harvesting and ecological alterations has collapsed its wild stocks and at present its status is reported as near threatened (Lakra *et al*., 2010; Ng *et al.,* 2010). To promote its conservation and stock enhancement, the ICAR-National Bureau of Fish Genetic Resources (ICAR-NBFGR), Lucknow, has prioritised this species for aquaculture diversification (NBFGR, 2011).

Primarily being a carnivorous fish, feed is an important bottleneck for the domestication of *O. bimaculatus. O. bimaculatus* received little attention in Indian aquaculture due to lack of sufficient seeds for stocking and knowledge gap in its farming (Debnath *et al.,* 2015). Deyoe and Tiemier (1998) rationalised that 30-35% dietary protein in supplementary feed is essential for better growth in catfishes. The present study evaluated the effect of protein concentrations of 30-35% on growth and production of *O. bimaculatus* in pond farming.

The study was conducted at the ICAR Research Complex for North-Eastern Hill (NEH) Region, Tripura Centre, Lembucherra using nine earthen ponds of uniform shape, size (0.03 ha), depth (1.5 m), basin conformation, contour and bottom type with proper inlet and outlet facility. The ponds were properly cleaned before initiating the experimental trial. Lime @250 kg ha^{-1}, raw cattle manure (RCM) @ 3000 kg ha^{-1} and single superphosphate (SSP) @ 30 kg ha^{-1} were applied for basal pond fertilisation (Santhosh *et al.,* 2007). A week later, when the water became bright/light green, pabda fingerings (1.6 g) procured from the hatchery, Department of Fisheries, Government of Tripura, were stocked @ 4000 ha^{-1}. There was no supplementary feeding for initial three days and fish were acclimatised in ponds with plankton. Feed supplementation started from 4^{th} day of stocking using the formulated feeds in powder form.

Three types of feeds (Feed-1, Feed-2 and Feed-3) were prepared for evaluation as per composition given

Table 1. Composition of the experimental feeds

Ingredients	Feed-1 (Conventional) (%)	Feed-2 (%)	Feed-3 (%)
Rice bran (RB)	50	32	24
Mustard oil cake (MOC)	50	28	36
Fish meal (FM)	-	30	30
Wheat flour (WF)	-	9	9
Vitamin-mineral premix (VMP)*	-	1	1
Total	100	100	100

*Each 5 kg contain Vitamin A-100,00,000 IU, Vitamin D_3-20,50,000 IU, Vitamin B_2-2.6 g, Vitamin E- 1750 IU, Vitamin K- 2 g, Calcium pantothenate -5 g, Vitamin B_{12}- 15 mg, Choline chloride-12% (w/w), Calcium- 1,700 g, Manganese-55 g, Iodine-2 g, Zinc-30 g, Iron-15 g, Copper-4 g and Cobalt-0.9 g

in Table 1. The feed ingredients *viz*., mustard oil cake (MOC), rice bran (RB), fish meal (FM), wheat flour (WF) and vitamin-mineral premix (VMP) were procured locally from Lembucherra, West Tripura. MOC was water-soaked overnight before using. The ingredients weighed to desired quantities using a laboratory balance, manually mixed and stored in plastic bags with labels. The feeds were analysed for crude protein (CP), crude lipid (CL), crude fibre (CF), ash, nitrogen free extract (NFE) and energy following methods as per AOAC (1984). The CP was 18.84% in Feed-1, 30.47% in Feed-2 and 35.24% in Feed-3 (Table 2). Fishes were fed @ 4% of body weight (BW) during initial 2 months, 3% of BW during next 2 months and 2% of BW during the last 2 months of culture. The feeding rate was adjusted based on monthly fish sampling and assuming 70-80% survival in biomass.

Intermittent fertilisation to maintain the pond fertility was carried out at 15 days intervals with RCM @500 kg ha^{-1}, urea @10 kg ha^{-1} and SSP @ 15 kg ha^{-1}. Intermittent liming was also done @100 kg ha^{-1} at 2-months intervals except at the onset of winter, when it was applied @200 kg ha^{-1}.

The water quality parameters were monitored at 15 daysinterval by sampling water from 10 cm below the water surface at 8.00-9.00 hrs in the morning. Temperature (^{0}C) was determined using thermometer, dissolved oxygen (DO) by Winkler's method, pH employing digital pH meter (Merck), transparency using Secchi disc and total alkalinity (TA) as per APHA (1998). Ammonia and phosphate levels were estimated using test kits (Spectroquant nova 60, Merck).

The pond sediments were randomly sampled from five locations in each pond at the beginning and end of the culture trial. Equal volume of sediment from each location was thoroughly mixed in a plastic bucket and a single composite sample was made. The composite sample was oven-dried at 60^{0}C, crushed using a mortar and pestle and sieved through a 0.85 mm screen for analysis of pH, available nitrogen (N), available phosphorus (P) and organic carbon (OC) following standard protocols.

Plankton samples were collected at 8.00-9.00 hrs at fortnightly intervals by filtering 50 l of water using bolting silk net (No. 25; mesh size - 34 µ) from five different locations of each pond to obtain a 50 ml sample, and analysed. The samples were preserved in plastic bottles with 10% neutral buffered formalin (NBF) and analysed qualitatively and quantitatively using Sedgwick-Rafter (SR) cell, haemocytometer and microscope (Leica DM1000) (Jhingran *et al*., 1969).

After 6 months of experimental feeding, all fish were harvested by repeated netting and survival was calculated. From the harvest, 5-10% of the fish biomass was individually weighed for total length and wet weight as well as average daily gain (ADG), specific growth rate (SGR), feed conversion ratio (FCR) and production was estimated. SGR was calculated using the formula, SGR (% per day) = (Ln final weight - Ln initial weight)/days of experiment) x 100. FCR was calculated as, weight gain/ feed given. Three fish were randomly sampled from each treatment and its flesh collected for proximate analysis following AOAC (1984). The data were analysed by one-way ANOVA at 5% level of significance employing SPSS software (v21.0).

The study demonstrated the effect of different practical diets on the growth and production of *O. bimaculatus* in fertilised ponds over 6 months period. The levels of dietary protein, estimated in the formulated feeds, were according to the experimental protocol planned while other constituents were almost uniform across the feeds (Table 2). Overall survival of the fish were almost uniform in all the trials (Table 3). This indicated that the ingredients used for feed formulation were of good quality and the protocol followed for feed preparation was near to standard protocols (Paul *et al.,* 2012). The graded increase in the dietary protein level in the feeds showed positive correlation with CL (r = 0.90) and energy (r = 0.97) and negative correlation with CF (r = -0.76), ash (r = -0.96) and NFE (r = -0.94) and sole compounding of RB and MOC led to highest CF and ash in the Feed (Rahman *et al.,* 1982).

Table 2. Proximate composition of the formulated feeds

Components (%)	Feeds		
	1	2	3
Moisture	12.04±1.06	10.12±0.97	10.07±0.60
Crude protein (CP)	18.84±0.57	30.47±0.54	35.24±0.17
Crude lipid (CL)	6.01±0.44	6.53±0.22	6.44±0.21
Crude fibre (CF)	9.52±0.24	8.36±0.37	8.83±0.35
Ash	18.81±0.70	13.23±0.95	13.20±0.36
Nitrogen free extract (NFE)	34.75±1.35	31.26±1.06	26.13±0.70
Energy (kcal g^{-1})	2.46±0.17	3.34±0.21	3.40±0.20

Table 3. Growth parameters of fish in the experimental groups

Parameters	Feed-1	Feed-2	Feed-3
Initial weight (g)	1.60±0.01	1.60±0.01	1.60±0.01
Final weight (g)	$64.8±3.06^a$	$72.00±2.00^b$	$74.10±2.50^b$
SGR (% day^{-1})	$3.9±0.45^a$	$4.01±0.24^a$	$4.04±0.21^a$
ADG (g)	$0.35±0.03^a$	$0.39±0.02^a$	$0.41±0.03^a$
FCR	$3.30±0.43^a$	$2.20±0.10^b$	$2.10±0.10^b$
Survival (%)	$76.2±3.14^a$	$75.4±0.90^a$	$75.2±1.65^a$
Production (kg ha^{-1})	$197.5±1.02^a$	$217.1±8.62^b$	$223.4±4.91^b$
Benefit cost ratio (BCR)	$1.76±0.15^a$	$2.20±0.10^b$	$2.13±0.05^b$

Figures in the same row having the same superscripts are not significantly different ($p \geq 0.05$)

The dietary protein requirement of fish is influenced by water quality, body size, stocking density and feeding rate. In this study, the range of water quality parameters recorded were: temperature 28.25-28.45^{0}C, transparency 45.82-46.13 cm, DO 5.10-5.12 mg l^{-1}, pH 7.45-7.5, TA 65.20-65.21 mg l^{-1}, ammonia-nitrogen 0.45-0.46 mg l^{-1} and phosphate-phosphorus 0.22-0.23 mg l^{-1} (Table 4). The range of soil parameters estimated were, pH 6.7-7.15, OC 0.42-1.34%, available N 1.75-38.22 mg% and available P 0.85-5.13 mg% (Table 5). Phytoplankton density varied from 7.65 to 7.83×10^3 cells l^{-1} and zooplankton density 2.39 to 2.44 x10^3 individuals l^{-1} (Table 6). Among the plankton samples analysed, Chlorophyceae was represented by the genera *Carteria, Platymonas, Chlamydomonas, Chlorogonium, Pyramimonas, Gonium, Pandorina, Eudorina, Volvox, Physocystium, Pleudorina, Phacotus* and *Dysmorphococcus;* Bacillariophyceae by *Cyclotella, Surirella, Melosira, Synura* and *Dinobryon*; Cyanophyceae/Myxophyceae by *Microcystis, Anabaena, Oscillatoria, Spirulina*, *Arthrospira* and *Raphidiopsis;* Euglenophyceae by *Euglena* and *Trachelomonas;* Dinophyceae by *Peridinum* and *Ceratium;* rotifers by *Brachionus, Asplanchna, Polyarthra* and *Keratella;* copepods by *Cyclops*, *Mesocyclops* and *Diaptomus* and Cladocerans by *Moina, Daphnia, Bosmina, Ceriodaphnia* and *Dipahanosoma*. The composition and abundance did not differ significantly between experimental ponds ($p \geq 0.05$) and results were similar to that of trials on different stocking densities by Debnath *et al*. (2015). The fingerlings were originally from the same stock, and all conditions including stocking density and feeding rate were also maintained uniform in all the experimental ponds. Water and soil parameters as well as plankton levels recorded were also found to be in the safe limits for fish culture.

Excess feeding and fertilisation can cause sedimentation and eutrophication and affect the pond

Table 4. Mean values of water quality parameters recorded in the experimental ponds

Parameters	Feed-1	Feed-2	Feed-3
Temperature (^{0}C)	$28.25±0.05^a$	$28.45±0.04^a$	$28.45±0.03^a$
Transparency (cm)	$45.82±0.15^a$	$46.13±0.22^a$	$46.04±0.26^a$
Dissolved oxygen (mg l^{-1})	$5.12±0.002^a$	$5.10±0.003^a$	$5.11±0.002^a$
pH	$7.45±0.02^a$	$7.50±0.005^a$	$7.46±0.002^a$
Total alkalinity (mg l^{-1})	$65.22±0.01^a$	$65.32±0.04^a$	$65.20±0.07^a$
Ammonia-nitrogen (mg l^{-1})	$0.45±0.005^a$	$0.45±0.002^a$	$0.46±0.006^a$
Phosphate-phosphorus (mg l^{-1})	$0.22±0.001^a$	$0.23±0.002^a$	$0.22±0.001^a$

Figures in the same row having the same superscripts are not significantly different ($p \geq 0.05$)

Table 5. Mean values of soil quality parameters recorded in the experimental ponds

Parameters	Feed-1	Feed-2	Feed-3
Soil pH			
Initial	6.73±0.11[a]	6.70±0.15[a]	6.72±0.12[a]
Final	7.13±0.05[a]	7.15±0.05[a]	7.08±0.13[a]
Organic carbon (%)			
Initial	0.42±0.15[a]	0.52±0.12[a]	0.52±0.12[a]
Final	1.32±0.12[a]	1.34±0.08[a]	1.33±0.12[a]
Available N (mg 100 g^{-1})			
Initial	1.75±0.08[a]	1.76±0.07[a]	1.81±0.06[a]
Final	32.50±5.15[a]	33.30±4.54[a]	38.22±1.22[a]
Available P (mg 100 g^{-1})			
Initial	0.86±0.05[a]	0.92±0.05[a]	0.85±0.05[a]
Final	4.23±2.25[a]	5.01±2.11[a]	5.13±2.14[a]

Figures in the same row having the same superscripts are not significantly different ($p \geq 0.05$)

Table 6. Mean values of plankton density recorded in the experimental ponds

Plankton groups	Feed-1	Feed-2	Feed-3
Phytoplankton ($\times 10^3$ cells l^{-1})			
Chlorophyceae	5.50±0.04[a]	5.48±0.05[a]	5.45±0.08[a]
Cyanophyceae	1.65±0.03[a]	1.56±0.05[a]	1.58±0.16[a]
Bacillariophyceae	0.56±0.04[a]	0.48±0.11[a]	0.54±0.02[a]
Euglenophyceae	0.12±0.011[a]	0.13±0.015[a]	0.14±0.008[a]
Total	7.83±2.44[a]	7.65±2.45[a]	7.71±2.42[a]
Zooplankton ($x10^3$ individual l^{-1})			
Rotifer	1.88±0.05[a]	1.92±0.02[a]	1.85±0.09[a]
Crustacea	0.54±0.35[a]	0.52±0.45[a]	0.54±0.41[a]
Total	2.42±0.94[a]	2.44±0.98[a]	2.39±0.92[a]

Figures in the same row having the same superscripts are not significantly different ($p \geq 0.05$)

environment (Santhosh and Singh, 2007). But, such situation was not noticed in this trial which indicated that the rate of feeding and fertilisation followed was adequate. Interestingly, all soil and water quality parameters followed similar trends of variation in all the ponds. This could be attributed to uniform shape, size, and basin conformation of the ponds (Murty *et al*., 1978). The increased concentration of nutrients at the end of culture was probably due to gradual deposition of metabolites and/or a portion of feeds or fertilisers used fortnightly (Jena *et al*., 2002).

The growth parameters of *O. bimaculatus* are presented in Table 3. Despite having less CP in Feed-1, the survival of fish was higher ($p \geq 0.05$) when compared with Feed-2 and Feed-3. This could be attributed to higher ($p \geq 0.05$) plankton production (Yurkowski and Tabacheck, 1979). The final fish weight was 11.11% higher in Feed-2 and 14.35% higher in Feed-3 when compared with Feed-1. Thus the SGR and ADG were higher ($p \geq 0.05$) in Feed-2 and Feed-3. Higher growth and weight gain in Feed-2 and Feed-3 could be attributed to higher dietary CP and energy levels in the feeds. Less protein, less energy and lack of vitamins led to poor growth in Feed-1. Patra (1994) also reported similar results in the climbing perch (*Anabas testudineus*). The higher production with Feed-2 (10%) and Feed-3 (13%) could be due to higher individual weight increment with these feeds. FCR was found to decrease with increased dietary CP of the feeds and it was significantly higher with Feed-1 when compared with Feed 2 and Feed-3. This indicated that nutrients of Feed-2 were better utilised by the fish as compared to nutrients of Feed-1. The excess CP of Feed-3 did not influence FCR, which could be due to improper protein-energy ratio in Feed-3 and deamination and wastage of protein through faeces (Kumar *et al*., 2011). The BCR was highest in Feed-2 followed by Feed-3 and it was lowest in Feed-1. This indicated farming with Feed-2 or Feed-3 is more profitable and sustainable.

The average CP content of the fish muscle varied from 13.96 to 14.16%, CL 4.40 to 4.47%, ash 4.24 to

4.40%, NFE 10.98 to 11.82% and moisture 64.8 to 66.3% (Table 7) and was found to be statistically insignificant among different treatment groups ($p \geq 0.05$). These results were also similar to the proximate composition of pabda reported by Debnath and Sahoo (2013). The results also indicated that the proximate composition of pabda was non-influential at the current level of CP supplementation (18.84-35.24%) in the feeds. Paul *et al.* (2012) also reported insignificant influence of dietary CP @28-38% on the carcass composition of *O. pabda* fry.

Table 7. Proximate composition of *Ompok bimaculatus* in response to different feeds

Components (%)	Feed-1	Feed-2	Feed-3
Moisture	65.66±0.75[a]	66.3±1.25[a]	64.8±0.70[a]
CP	14.03±0.20[a]	14.16±0.97[a]	13.96±0.32[a]
CL	4.43±0.20[a]	4.40±0.21[a]	4.47±0.22[a]
Ash	4.40±0.20[a]	4.31±0.16[a]	4.24±0.12[a]
NFE	11.76±0.65[a]	10.98±0.46[a]	11.82±0.61[a]

The results of the experimental trials clearly indicated that inclusion of dietary protein influences the growth and production of *O. bimaculatus*. Growth was found to be 11.11% higher with 30% CP and 14.35% higher with 35% CP when compared with conventional RB and MOC mixture. Production was 10% higher with 30% CP and 13% higher with 35% CP as compared to conventional feed. Difference in growth and production recorded with CP levels of 30 and 35% was found to be statistically not significant and hence CP level can be restricted to 30% for sustainable pabda farming in fertilised pond systems.

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