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Effect of dietary incorporation of natural attractants on growth and survival during seed rearing of Indian butter catfish, *Ompok bimaculatus*

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Abstract

Aim : The objective of the study was to assess the effect of dietary incorporation of different feed attractants viz. tubifex, earthworm and fish meal on growth and survival of *O. bimaculatus* (pabda fish) fry.

Methodology : Fifty numbers of fifteen days weaned fry (0.105 ± 0.008 g; 2.14 ± 0.14 cm) were stocked into each aerated aquariums (30.0 x 15.0 x 15.0 cm) following a completely randomized design (CRD) consisting of four treatments including the control with three replicates each. Four iso-nitrogenous purified diets were prepared including three treatment diets with attractants (5%) by replacing an appropriate amount of casein and cellulose and fed to the fishes twice daily.

Results : Results from the study showed that growth of fry as measured by final weight, weight gain, mean daily weight gain and specific growth rate of fries were significantly ($p < 0.05$) higher in tubifex supplemented group and lower in control group. Survival was significantly higher in tubifex supplemented group ($42.66 \pm 1.3\%$) and lower in control group. Moreover, other yield parameters such as total biomass, condition factor and performance index also followed the same trend as survival. Further, higher protease activity was found in control group (2.233 ± 0.038 units $\text{mg protein}^{-1} \text{min}^{-1}$) followed by earthworm (0.354 ± 0.031 $\text{mg protein}^{-1} \text{min}^{-1}$) supplemented group ($p < 0.05$). The lipase and amylase activity were higher ($p < 0.05$) in tubifex and control groups, respectively.

Interpretation : It could be concluded that the dietary supplementation of tubifex at 5% inclusion level could be a promising aquaculture feeding strategy for pabda fish due to its rich nutrient content, increased feeding stimulation and more palatability.

Key words: Digestive enzyme, Earthworm, *Ompok bimaculatus*, Tubifex, Seed rearing

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Introduction

Freshwater aquaculture sector has witnessed an encouraging growth in the past two decades by putting various indigenous catfishes into the culture systems due to its high profitability (Jayasankar, 2012). There are many catfishes which have numerous potential for freshwater aquaculture diversification. Of recent, Indian butter catfish, *Ompok bimaculatus* (Family: Siluridae), locally called as “Pabda” has gained importance as a promising aquaculture candidate due to its good taste, excellent nutritional profile, soft bony structure, rich lipoprotein content and high market value (Biswas et al., 2018). However, in recent times, the fish is under severe threat due to indiscriminate use of pesticides and weedicides, loss of breeding grounds and overfishing of brood fish in wild, resulting in listing the under threatened category (Ng et al., 2010).

Although, the successful induced breeding of species had a good beginning in India (Chakrabarti et al., 2012; Debnath et al., 2013), however, the fish did not receive much attention in farming due to inadequate seed availability, which was challenged by lower survival rate during seed rearing. The major reason for lower survival is attributed to cannibalism, inadequate larval food, low acceptability towards artificial diet, and improper feeding regime, during larval rearing phase of the species (Chakrabarti et al., 2012). The incidence of aggression and subsequent cannibalism in seed rearing leads to the heterogeneous growth of fry which further magnifies cannibalism. The current research has focused on encouraging the feed consumption by including of different feed attractants which in turn improve the survival and growth. It has been observed that better diet acceptability due to feed attractants facilitate early weaning of fish seed (Oliveira and De Cyrino, 2004; Gaber, 2005; Rawat et al., 2018). Natural attractants are those which exhibit attractant or feeding stimulant properties and are present in the impure or crude form.

The use of natural attractants such as mussel flesh, shrimp meal, oligochaete worms, fish protein hydrolysates, encourages quicker and complete feed intake, reducing levels of uneaten or wasted feed which in turn results in improved feed conversion and water quality of the rearing environment (Lee and Myers, 1997; Shankar et al., 2008). There have been various efforts towards the improvisation of seed rearing performance of fishes with dietary incorporation of different natural attractants such as lumbricid worms (Tacon, 1983), silkworm pupae powder (Akiyama et al., 1984), krill hydrolysate (Kolkovski et al., 2000), blue mussel (Nagel et al., 2013) etc. Although a substantial amount of research has been conducted on the identification of feed attractant for fish, there are only a few cases where feed attractant have been applied in starter aqua feed. Feed attractant can be of immediate practical importance in enhancing the acceptance of artificial feeds by first feeding larvae and fry. Keeping all this in view, the present study aimed to determine the effect of dietary supplementation of different natural attractants on growth, survival and production of pabda, *O. bimaculatus* fry.

Materials and Methods

Experimental animals and design: The present study was conducted for a period of 30 days at the Wet Laboratory, College of Fisheries, Central Agricultural University, Lembucherra, Tripura, India. Young ones of *O. bimaculatus* were fed with zooplanktons up to 11dph (days post hatching). Thereafter, weaning was done with artificial dry diet up to 15 dph. The resulting fry were used for the experiment. Twelve aquariums (30.0 x 15.0 x 15.0 cm) were filled with 25 l of water and provided a continuous aeration. All the aquariums were stocked with fifty fry (0.105 ± 0.008 g; 2.14 ± 0.14 cm) in each and were assigned to four dietary treatment groups namely control, tubifex, earthworm and fish meal with three replicates, each following a completely randomized design (CRD). The fish were fed twice daily at 05.00 and 18.00 hr under a normal light regime (light/dark: 12/12 hr). The water quality parameters such as temperature, pH, dissolved oxygen (DO), free carbon dioxide (CO₂), carbonate hardness, ammonia-N and nitrate-N were monitored.

Experimental diets: Four iso-nitrogenous purified experimental diets were prepared according to the formulation given in Table 1 with supplementation of three different attractants, i.e., tubifex, earthworm and fish meal at 5% level by replacing appropriate amount of casein and cellulose. Initially, a desired quantity of cleaned earthworm and/or tubifex was taken in a 5 l. beaker and kept for 24 hr to empty the alimentary canal. Thereafter, the worms were washed thoroughly and wet-grounded with a blender. The product was then freeze-dried in a lyophilizer and finally the resultant freeze dried flakes were ground into powder form. Weighed quantities of different feed ingredients (except vitamin-mineral mix) were sieved through a 60 mesh screen and homogenized by blending thoroughly in a feed mixture. The required amount of fish oil was added to the ingredients and made into dough with appropriate amount of water. The dough was then cooked in steam for 30 min and then allowed to cool. After cooling, vitamin-mineral mix was thoroughly mixed in a blender. Thereafter, the feeds were again freeze dried and stored in a refrigerator at 4°C until further use.

Growth study: To evaluate the effects of dietary supplementation of natural attractants on growth and quality of Pabda seed, the biometric parameters such as weight gain, length increment, specific growth rate, Fulton's condition factor and performance index were assessed.

Survival percentage: Survival rate was measured at the end of the experiment by counting the number of fish in tank and calculated as Survival (%) = $100 \left[\frac{\text{Number of surviving fish}}{\text{Total number of larvae stocked}} \right]$

Digestive enzyme analysis: Three fishes from each treatment were anesthetized using clove oil @ 50 µl l⁻¹ of water followed by dissection whereas intestines were obtained carefully for preparation of 5% homogenate with chilled sucrose solution (0.25M). The homogenate was centrifuged at 5000 rpm for 15 min

at 4°C, and the resultant supernatant was stored in glass vials at -20°C until digestive enzymes were assayed. The protein content of intestine was estimated by Lowry's method (Lowry *et al.*, 1951). The activity of protease, lipase and amylase were estimated following the methods described by Drapeau (1974), Cherry and Crandall (1932) and Rick and Stegbaur (1974), respectively.

Proximate analysis of diets: The proximate composition of the experimental diets was analyzed following the standard methods (AOAC, 2005). Briefly, the moisture content of the diets were determined by drying a 2g sample to constant weight in an oven at 105±3°C.; crude protein (CP) was calculated indirectly from the analysis of total nitrogen (crude protein, Nx6.25) by the Kjeldahl method (2200 Kjeltac auto distillation, Foss Tecator, Sweden) and Ether extract (EE) was measured by Soxtec (1045 Soxtec Extraction Unit, Tecator, Sweden), using diethyl ether (boiling point, 40–60°C) as a solvent. Total ash content was evaluated from weighed samples in a porcelain crucible placed in a muffle furnace (Nutronics, New Delhi, India) at 600°C for 5-6 hr and crude fiber (CF) content by

acid digestion (1.25%), followed by alkali digestion (1.25%) in a fiberplus apparatus. The nitrogen free extract (NFE) was calculated as $NFE = 100 - (CP + EE + CF + Ash)$.

Statistical analyses: Response data were analyzed by one-way analysis of variance (ANOVA) and the significant difference between the treatments was determined by Duncan's Multiple Range Test using SPSS (Version 16.0, SPSS Inc., Chicago, IL, USA). The results were expressed as mean ± S.E. The level of significance employed was 0.05.

Results and Discussion

The level of crude protein of the experimental diets was found close to the formulated value (50%). In the study, significantly ($p < 0.05$) higher ether extract was found in tubifex supplemented diet, followed by earthworm supplementation. Further, the fibre content was significantly ($p < 0.05$) higher in earthworm supplemented diet and lower in fish meal supplemented diet. During the experimental period, water

Table 1: Ingredient proportion and proximate composition of experimental diets supplemented with natural attractants fed to *O. bimaculatus* fry for 30 days

Ingredients	Inclusion level (%)				p-value
	Control diet	Tubifex supplemented diet	Earthworm supplemented diet	Fish meal supplemented diet	
Casein	55	51.99	52.52	52.33	-
Corn starch	15	15	15	15	-
Fish oil	8	8	8	8	-
Vitamin mineral Mix ¹	8	8	8	8	-
Guar gum	4	4	4	4	-
Cellulose	10	8.01	7.48	7.67	-
Tubifex	-	5	-	-	-
Earthworm	-	-	5	-	-
Fish Meal	-	-	-	5	-
Proximate composition					
Moisture	4.23±0.03	4.20±0.003	4.21±0.01	4.20±0.003	0.610
Crude protein (CP)	49.80±0.2	49.20±0.5	49.40±0.3	49.23±0.2	0.606
Ether extract (EE)	8.24±0.005 ^a	8.43±0.003 ^d	8.32±0.006 ^c	8.30±0.003 ^b	0.000
Fibre	4.16±0.06 ^b	4.6±0.03 ^c	4.8±0.03 ^d	3.87±0.01 ^a	0.000
Ash	8.40±0.006	8.45±0.006	8.46±0.005	8.47±0.01	0.620
Nitrogen free extracts (NFE)	29.30±0.10	29.20±0.60	28.90±0.30	30.10±0.20	0.213
Digestible energy ² (kcal 100 g ⁻¹)	390.70±0.2 ^c	389.60±0.09 ^b	388.40±0.14 ^a	392.10±0.07 ^d	0.000

*Values are mean of six replicates ± S.E.; Different superscripts in the same row signify statistical differences ($p < 0.05$)

¹Vitamin-mineral mix (Minerex Forte) (quantity/1kg): Vitamin A-20,00,000 IU; Vitamin D3-4,00,000 IU; Vitamin E-300 I.U.; Vitamin B12- 2.4 mg; Vitamin B2-0.8 g; Vitamin K-30.4 g; Calcium D panthothenate-1 g; Choline chloride-60 gm; Ca- 300 g; Mn- 11 g; Fe- 3 g; Cu-0.8 g; Co- 180 mg; Se-40 ppm; Niacinamide-4 gm; Zn- 2128 mg; Tri sodium citrate as chelating agent; Approximate overages and antioxidants added

²Digestible energy (kcal 100 g⁻¹) = (CP% × 4) + (EE% × 9) + (NFE% × 4)

temperature ranged from 26.4 to 28.6°C; dissolved oxygen levels were above 10 mg l⁻¹; total alkalinity ranged between 25.0 and 38.0 mg CaCO₃ l⁻¹; total hardness ranged between 24.0 and 34.0 mg CaCO₃ l⁻¹; carbon dioxide and ammonia-nitrogen (NH₃-N) ranged between 0.02–0.05 and 1.0–3.0 mg l⁻¹, respectively; pH fluctuated between 7.0 and 7.9. The water quality parameters did not show variation among the treatments, possibly due to the periodic exchange of water with matured water from the same sources and management measures adopted during the study. These values were found within the normal range of pabda seed rearing (Chakrabarti et al., 2012; Rawat et al., 2018) which indicated prevalence of suitable growing environment for the species.

Growth, as indicated by final weight, weight gain and specific growth rate of *O. bimaculatus* fry, was significantly higher in tubifex diet group, followed by fish meal diet and earthworm supplemented group, however, the lowest was observed in the control diet group (Table 2). The higher growth in tubifex supplemented group (0.66±0.005 g) might be attributed to the stimulation of feeding behaviour of *O. bimaculatus* fry due to more palatability, and consequently increased its acceptance by fish. Oligochaetes such as tubifex and earthworm have been known to

contain food attractants for several catfishes (Hashim et al., 1993). In concurrent to this finding, better growth at tubifex diet was also observed by several researchers in various fish species such as *Clarias macrocephalus* (Hashim et al., 1993), *Pangasius bocourti* (Hung et al., 1999), *Ompok pabda* (Chakrabarty and Chakrabarti, 2005), *Chitala chitala* (Sarkar et al., 2006), *Cyprinus carpio* (Mahfuj et al., 2012) and *Clarius batrachus* (Mandal et al., 2018). On the other hand, the increased growth by fish meal supplementation might be due to the fact that fish meal is reported to contain higher amino acid profile (IAFMM, 1970), which contributed to the better growth of pabda fry in our study. Further, the positive effect of fish meal on growth and food acceptance has been reported in other species such as, *Scophthalmus maximus* (Oliva-Teles et al., 1999), Atlantic salmon (Pike et al., 1990; Anderson et al., 1993), Atlantic halibut (Aksnes and Mundheim, 1997) and *Dicentrarchus labrax* (Peres and Oliva-Teles, 2007). Tacon et al. (1983) reported that the rainbow trout fed on earthworm meal (*E. foetida*) protein did not yield any encouraging growth. Similarly, Hilton and Reynolds (1983) also observed a growth depression in trout fed with earthworm meals which probably attributed to lack of some unidentified essential components in the diet. However, Mahanta et al. (2016) found an encouraging result with earthworm supplemented diet and

Table 2 : Growth parameters in different experimental groups fed with different natural feed attractants

Parameters	Control diet	Tubifex supplemented diet	Earthworm supplemented diet	Fish meal supplemented diet
Initial length (cm)	2.14±0.14	2.14±0.14	2.14±0.14	2.14±0.14
Initial weight (g)	0.105±0.008	0.105±0.008	0.105±0.008	0.105±0.008
Initial stock (nos.)	50	50	50	50
Final length (cm)	4.1±0.05	4.3±0.05	4.2±0.11	3.7±0.33
Final weight (g)	0.35±0.003 ^a	0.66±0.005 ^c	0.42±0.001 ^b	0.46±0.0008 ^b
Weight gain (g)	0.21±0.003 ^a	0.50±0.005 ^c	0.32±0.001 ^b	0.38±0.0008 ^b
Body weight gain (%)	236.5±3.1 ^a	528.5±5.4 ^c	305.7±1.09 ^b	339.3±0.8 ^b
Length increment (cm)	1.96±0.05	2.1±0.05	2.06±0.1	1.6±0.3
Specific growth rate (% d ⁻¹)	7.62±0.009 ^a	8.25±0.008 ^c	7.81±0.002 ^b	7.89±0.001 ^b
Mean daily weight gain (%)	0.95±0.01 ^a	2.13±0.02 ^c	1.23±0.004 ^b	1.3±0.003 ^b

*Overall mean value having different superscript in the same row shows significance difference (p<0.05); a, b, c, d denotes significance differences between different treatments; values are mean ± S.E.

Table 3 : Mean yield parameters in different experimental groups fed with different natural feed attractants

Parameters	Control diet	Tubifex supplemented diet	Earthworm supplemented diet	Fish meal supplemented diet
Survival (%)	28.00±1.15 ^a	42.66±1.33 ^c	30.66±1.7 ^{ab}	34.66±2.66 ^b
Total biomass (g)	4.95±0.24 ^a	14.08±0.54 ^c	6.53±0.38 ^b	7.99±0.63 ^b
Condition factor (K)	1.4±0.02 ^a	2.8±0.03 ^c	1.70±0.05 ^b	1.70±0.15 ^b
Performance index	0.26±0.01 ^a	0.91±0.03 ^d	0.37±0.02 ^b	0.47±0.03 ^c

*Overall mean value having different superscript in the same row shows significance difference (p<0.05); a, b, c, d denotes significance differences between different treatments; values are mean ± S.E.

Table 4 : Digestive enzyme activity (units mg protein⁻¹ min⁻¹) of *O. bimaculatus* fry reared in different experimental groups

Parameters	Control diet	Tubifex supplemented diet	Earthworm supplemented diet	Fish meal supplemented diet
Protease	2.233±0.038 ^c	0.198±0.017 ^a	0.354±0.031 ^b	0.245±0.010 ^a
Lipase	0.36±0.0001 ^a	2.416±0.236 ^c	1.893±0.606 ^{bc}	1.190±0.224 ^{ab}
Amylase	0.634±0.006 ^c	0.067±0.003 ^a	0.101±0.008 ^b	0.064±0.004 ^a

*Overall mean value (±SE) having different superscript in the same row shows significance difference ($p < 0.05$); a, b, c denotes significance differences between different treatments

recommended earthworm diet for Rohu fry. Tuan *et al.* (2016) also observed earthworm as a suitable replacement for fish meal in the diet of common carp which is in agreement with the present study.

Other yield parameters such as survival, total biomass, condition factor and performance index are presented in Table 3. In the study, significantly higher survival was observed in tubifex supplemented diet (42.66±1.33%) and lower in the control diet (28.00±1.15%). The higher survival in tubifex diet group might be due to the presence of tryptophan which showed the calming effect on the aggressive behaviour of pabda fries (Hoglund, 2005; Kumar *et al.*, 2017, Rawat *et al.*, 2018). Moreover, the tryptophan content of tubifex is higher than that of earthworm and fish meal (Herawati *et al.*, 2016). In addition, some earlier studies also reported the positive effect of tubifex on survivability on various fish species such as *Rhombosolea tapirina* (Hart *et al.*, 1996), *Pangasius bicourti* (Hung *et al.*, 2002), *Ompok pabda* (Chakrabarty *et al.*, 2007), *Ompok bimaculatus* (Chakrabarti *et al.*, 2012; Malla and Banik, 2015) and *Clarius batrachus* (Mandal *et al.*, 2018). The results of the study showed significantly higher condition factor in tubifex supplemented diet group, however, the lowest was observed in control diet group ($p < 0.05$), which might be due to the better palatability of tubifex supplemented diet which gives better food intake, and consequently improved the condition factor. The study also showed a significantly higher ($p < 0.05$) total biomass and performance index in tubifex supplemented group, followed by fish meal supplemented group, might be due to higher survival rate and final weight in case of tubifex supplemented group than others, as evidenced from the results of this study.

The digestive enzyme activities (Table 4) indicated a higher protease activity in the control group (2.233) followed by earthworm supplemented group, (0.354) whereas the lowest was found in both tubifex and fishmeal supplemented diets ($p < 0.05$). The significantly higher protease activity in control group may be attributed to lower feed intake which in turn increases the capacity of protein digestion (Rawat *et al.*, 2018). Moreover, the cannibalism was aggravated in control and earthworm supplemented group, thereby consumption of their own siblings may lead to higher protease activity. These findings corroborates with the study of Bolasina *et al.* (2006). Lipase content mainly depends on the nature of food, dietary lipid requirements, developmental stage and the species (Sharma and Chakrabarti, 1999; Rawat *et al.*, 2018). Lipase activity was significantly

($p < 0.05$) lower in control and fish meal supplemented group which might be due to the presence of less dietary lipid content in these diets than that of tubifex and earthworm supplemented diets. Further, significantly higher ($p < 0.05$) amylase enzyme activity was observed in control diet (0.634±0.006 mg protein⁻¹) followed by earthworm supplemented diet, whereas no significant response was recorded in tubifex and fish meal supplemented diets. This attributed to the higher level of dietary carbohydrate (cellulose) level in control diet, which is in agreement with the reports of Rawat *et al.* (2018) in pabda fry.

From the foregoing discussion, it can be concluded that the dietary supplementation of tubifex at 5% level improved the growth performance, as well as survivability during pabda fry rearing under indoor condition. Considering the fact that for seed rearing of many fishes, survivability of seed is more important than growth as economic returns through seed material is high, supplementation of tubifex at 5% level can be suggested to end users. Overall, this finding will give directions for further research on the insight mechanisms of the effects of natural attractants on seed rearing through molecular and biochemical tools.

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