www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences 18: 491-499 (2018) DOI: 10.4194/1303-2712-v18_3_15

RESEARCH PAPER

Effect of Dietary Incorporation of Chemo-Attractants on Growth and Survival during Seed Rearing of *Ompok bimaculatus* (Bloch)

Priya Rawat Pradyut Biswas[1,*], Arun Bhai Patel[1], Himadri Saha[1]

[1] College of Fisheries, Central Agricultural University (Imphal), Lembucherra, Agartala–799 210, Tripura, India.

* Corresponding Author: Tel.: +91.801 4496141 ; Fax: +91.381 2865291;
E-mail: pradyutbiswas@gmail.com

Received 12 May 2017
Accepted 31 July 2017

Abstract

A 30-days experiment was conducted to evaluate the efficacy of four chemo-attractants viz. DL-alanine, betaine, L-tryptophan (TRP) and inosine monophosphate (IMP) in the diet of *Ompok bimaculatus* during fry rearing. Fifty numbers of fifteen days weaned fry (0.0739±0.008 g; 2.32±0.06 cm) were stocked into each aerated aquariums (30.0 x 15.0 x 15.0 cm) following a completely randomized design (CRD) consisting of five treatments including control with three replicates each. Five iso-nitrogenous purified diets were prepared including four treatment diets with attractants (2%) replacing the cellulose and fed to the fishes twice a day. The highest growth was observed in the treatment fed with betaine supplemented diet followed by inosine monophosphate whereas, no significant difference was observed among control, DL-alanine and L-tryptophan supplementation. The highest survivability was found in L-tryptophan supplemented diet (48.66±2.4%) followed by betaine, DL-alanine, control and lowest was found in inosine monophosphate treatment (32.00±2.0). It has also observed that there was significant difference (P>0.05) in survival between L-tryptophan and betaine supplemented diets fed groups. It is concluded that the dietary tryptophan supplementation could be a promising aquaculture management strategy for carnivorous fish as it showed significantly better survival without affecting the growth.

Keywords: *Ompok bimaculatus*, L-tryptophan, betaine, digestive enzyme, inosine monophosphate.

Introduction

Aquaculture of various catfish in India is widely spreading as an organized industry due to its lucrative return of investment (Jayasankar, 2012). Among catfishes, the butter catfish, *Ompok bimaculatus* (Bloch, 1794) popularly known as Pabda, is recently gaining importance as a promising aquaculture candidate owing to its good taste, excellent nutritional profile, soft bony structure, rich lipo-protein content and high market value especially in the entire East and North East India (Banik, Goswami, & Malla, 2012). But nowadays, this fish has become scarce due to modification of habitat, indiscriminate use of pesticides and weedicides, loss of breeding grounds and overfishing of brood fish etc. Due to these reasons, the species is on the verge of extinction and has been listed under threatened category (Ng, Tenzin, & Pal, 2010). However, the culture of catfishes requires constant supply of good quality fingerlings. Main challenge for seed production is higher larval mortality due to inappropriate rearing practices. Aggression and cannibalism contributes significantly to mortality during seed production, even where conditions appear to be ideal (Hecht & Pienaar, 1993; Baras & Jobling, 2002). The occurrence of aggression and consequent cannibalism in seed rearing can be attributed to intrinsic genetic effects that result in heterogeneous growth of larvae. Because extrinsic factors can be manipulated, current aquaculture research has focused on managerial aspects such as encouraging the feed consumption by incorporation of feed attractants which in turn improve the survival and shorten the production intervals. The feed attractants are specific compounds or ingredients added to the feed to enhance the diet palatability and consequently, its acceptability by fish (Smith, Tabrett, Barclay, & Irvin, 2005). As a result of the improvement in the diet acceptability, the fish can adapt earlier to artificial dry diet during the weaning period and attain a higher overall feed consumption and growth rate (Tandler, Berg, & Mackie, 1982; Kolkovski, Arieli, & Tandler, 1997; de Oliveira & Cyrino, 2004; Gaber, 2005). Moreover, the use of attractants promotes quicker feed intake, minimizing the time that the feed remains in water and so preventing the deterioration of the water quality (Shankar, Murthy, Pavadi, & Thanuja, 2008).

Further, attractants provide additional nutrients for protein and energy metabolism (Papatryphon & Soares, 2001). Feeding processes of fish is governed by the chemo attraction and chemo stimulation which facilitate the initial location (olfactory) and final consumption (gustatory response) of food. Considering the importance of various behavioral components of fish, it is logical to assume that by adding attractants in the feed, fish can be attracted towards in a shorter period of time, creating the condition for faster ingestion. There have been various attempts towards improvisation of seed rearing performance of fishes with dietary supplementation of different attractants such as L-amino acids like glycine, alanine, proline, betaine, tryptophan (TRP), histidine (Hughes, 1991; Kolkovski, *et al*., 1997; Sola & Tongiorgi, 1998) and nucleotides like Adenosine monophosphate (AMP), Inosine monophosphate (IMP), Uracil monophosphate (UMP) and ADP (Kiyohara, Hidaka, & Tamura, 1975; Mackie & Adron, 1978; Ishida & Hidaka, 1987). Keeping the beneficial effect of dietary attractants, this study aimed to evaluate the effect of different chemo-attractants during seed rearing of Pabda (*Ompok bimaculatus*) which is one of the most interesting and promising fish species for diversification in freshwater aquaculture in the Indian sub-continent.

Materials and Methods

Experimental Animals and Design

The present study was conducted for 30 days at the College of Fisheries, Central Agricultural University, Lembucherra, Tripura, India aiming to evaluate four different chemo attractants on seed rearing performance of *Ompok bimaculatus*. After successful breeding, young ones are fed with natural food like planktons, chopped tubifex up to 11 dph (day post hatch). Thereafter, weaning has been done with artificial dry diet upto 15 dph. The resulting fry has been collected and stocked for the experiment. Fifteen aquariums (30.0 x 15.0 x 15.0 cm) were used for the experiment and filled with 25 l of water with aeration before introducing the fry. Each of the fifteen experimental aquariums was stocked with fifty fry (0.0739±0.008 g; 2.32±0.06 cm) following a completely randomized design (CRD) consisting of five treatments (feeds) with three replicates each. Round the clock aeration was provided to all the tanks. The daily water exchange amounted to 50% of the total unit volume with chlorine-free bore well water. The water quality parameters *viz*, temperature, pH, dissolved oxygen (DO), free carbon dioxide (CO_2), carbonate hardness, ammonia-N and nitrate–N were recorded every week following standard method (APHA *et al*., 1998) to check the water quality. The fish were fed twice at 0500 and 18.00 h. under a normal light regime (light/dark 12/12 h) and sampling of fish was done every after 15 days.

Experimental Diets

Five iso-nitrogenous purified diets (one control and four treatment diets) were formulated and prepared and the composition of the diets is given in the table 1. Fat free casein (HiMedia Laboratories Pvt. Ltd.) was used as the protein source, whereas cod liver oil was used as lipid source, corn starch and cellulose as carbohydrate source. Treatment diets are supplemented with four different attractants (Betaine, DL-alanine, L-tryptpphan and inosine mono phosphate) at 2% level by replacing cellulose. Weighed quantities of different ingredients were mixed (except vitamin-mineral mix and attractants) thoroughly, made into dough with appropriate amount of water, cooked in steam for 30 min and then cooled. After cooling, vitamin-mineral mix and attractants was thoroughly mixed with the dough in a blender. Thereafter, the feeds were freeze dried and stored 4^{o}C until use.

Growth Study

To assess the effects of dietary chemo attractants on growth and condition, all the fry in the tank were counted and a sub-group of 20 randomly selected fish from each treatment were individually measured to the nearest 0.1 mg. For monitoring the growth performance of the larvae, the different parameters were calculated based on the following formulae:

Mean weight gain (g) = Final weight – initial weight

Length increment (cm) = Final length – initial length

Body Weight gain (BWG)% =100 [(final weight – initial weight)/ initial weight]

Specific growth rate (SGR)% = 100[{ln (final weight) – ln (initial weight)}/experimental period]

Mean daily Weight gain (%) = 100 [(total final weight – total initial weight)/ days of experiment]

Fulton's condition factor (K) = (final weight/ final length ^{3})

Total biomass (g) = final number of fish × mean final weight

Performance index (PI) = Survival rate (%) [(Final mean body weight –Initial mean body weight)/ days of experiment]

Survival Percentage

Survival percentage was calculated at the end of the experiment by counting the number of fish in tank and is calculated as follows:

Survival (%) = 100 [(Number of surviving fish/ Total number of larvae stocked)

Digestive Enzyme Analysis

Preparation of Tissue Homogenate

Intestines were collected for preparation of 5% homogenate with chilled sucrose solution (0.25M). The homogenate was centrifuged at 5000 x g for 15 minutes at cold centrifuge at 4^0C then collected supernatant was stored in a sample vial at -20^{o}C until digestive enzymes was assayed. The intestines used for digestive enzyme assay with respect to each dietary treatment were in fresh condition and thereafter different enzymes were analyzed using standard curve.

Protein Estimation

Estimation of protein in intestine was carried out by Lowry's method (Lowry, Rosebrough, Farr, & Randall, 1951). Tissue homogenate 0.1 ml was taken and precipitated using 1 ml of 10% TCA. The protein residue was obtained by discarding the supernatant produced after centrifugation at 5000 rpm for 20 minutes. The residue was dissolved in 0.5 ml of 0.1 N NaOH. An amount of 0.1 ml of the dissolved protein residue was used for further analysis. Alkaline copper sulphate 5 ml was added and left for 10 minutes in the dark. Reading was taken at 660 nm against the blank. Bovine serum albumin was used as standard.

Protein (mg per weight of tissue) = [{(OD Test/OD standard) x (concentration of standard)} / (Sample taken)]

Protease Activity

It was determined by the casein digestion method (Drapeau, 1974). One unit of enzyme activity was defined as the amount of enzyme need to release acid-soluble fragments equivalent to 0.001A, 280 per minute at 37^0C and pH 7.8. The enzymes reaction mixture consisted of 1% casein in 0.05 M of tris HCl buffer (pH 7.8) and incubated for 5 minutes at 30^0C. Then tissue homogenate was added. Ten minutes later, reaction was stopped by adding 10% TCA and the whole content was filtered. The reagent blank was made by adding tissue homogenate just before

Table 1. Formulation and proximate composition (% dry matter) of the experimental diets fed to *O. bimaculatus* fry for 30 days

Components	Control diet	Betaine diet	Alanine diet	Tryptophan diet	Inosine mono phosphate diet	*p-value*
Casein	55	55	55	55	55	-
Corn starch	15	15	15	15	15	-
Fish oil	8	8	8	8	8	-
Vitamin-mineral mix[1]	8	8	8	8	8	-
Guar gum	4	4	4	4	4	-
Cellulose	10	8	8	8	8	-
Betaine	-	2	-	-	-	-
DL-Alanine	-	-	2	-	-	-
L-Tryptophan	-	-	-	2		-
Inosine mono phosphate	-	-	-	-	2	-
			Proximate Composition (mean ± SE)			
Moisture	6.41±0.005	6.40±0.01	6.41±0.008	6.36±0.03	6.36±0.03	0.386
Crude protein (CP)	49.83±0.20	49.3±0.50	49.5±0.05	49.3±0.50	49.4±0.20	0.837
Ether extract (EE)	8.20±0.001	8.21±0.006	8.21±0.001	8.23±0.001	8.22±0.003	0.080
Fibre	4.29±0.003^b	4.11±0.005^a	4.10±0.001^a	4.11±0.003^a	4.10±0.003^a	0.000
Ash	8.24±0.020	8.34±0.04	8.36±0.03	8.34±0.04	8.27±0.02	0.158
Nitrogen free extracts (NFE)	29.6±0.20	30.03±0.50	29.8±0.06	30.0±0.50	29.9±0.20	0.910
Digestible energy[2]	390.87±0.12^a	391.27±0.19^{ab}	391.18±0.13^{ab}	391.3±0.18^{ab}	391.56±0.08^b	0.090

Different superscripts in the same row signify statistical differences (P<0.05) (mean ± S.E.) (n = 6)

[1]Vitamin-mineral mix (Minerex Forte) (quantity/1kg): Vitamin A-20,00,000 IU; Vitamin D_3-4,00,000 IU; Vitamin E-300 I.U.; Vitamin B_{12}-2.4 mg; Vitamin B_2-0.8 g; Vitamin K_3-0.4 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.

[2]Digestible energy (kcal/100 g) = (CP% x 4) + (EE% x 9) + (NFE% x 4)

stopping the reaction and with no incubation. Then reading was taken at 280 nm.

Protease activity (units mg protein^{-1} min^{-1}) = [OD test / {(10 min) x (mg of protein in the sample)}]

Lipase Activity

It was assayed as described by Cherry and Crandall (1932). The volume (ml) of N/20 two test tube labelled as test (T) and control (C) were taken and into each test tube 3 ml of distilled water and 1 ml homogenate was added to both tubes. The control tube was placed in boiling water for 5 minutes at 100^0C and cooled. This serves to inactivate the lipase in the control. Then 0.5 ml phosphate buffer solution (pH 7) and 2 ml of olive oil emulsion were added to both the tubes, shaken well and incubated at 37^{o}C for 24 hours. Then 3 ml of 95% alcohol and 3 drops of phenolphthalein indicator solution were mixed. Each of the tube was titrated against 0.05 N NaOH upto the appearance of permanent pink colour. The volume (ml) of N/20 NaOH required for 100 mg intestinal tissues in the experimental tube minus the volume (ml) of N/20 NaOH solution required for the same amount of intestinal tissue in the control tube represented the units of intestinal lipase activity per g tissue. One unit will hydrolyse 1.0 micro equivalent of fatty acid from a triglyceride in 24h at pH 7.7 at 37^0C.

Lipase (Units per ml enzyme) = [{(volume of NaOH) x (M of NaOH) x (1000) x (2) x (dilution factor)} / volume (ml) of enzyme used]

Lipase (units mg protein^{-1}) = (units enzyme ml^{-1}) / (mg protein ml^{-1})

Amylase Activity

Amylase activity was estimated using dinitro-salicyclic-acid (DNS) method (Rick & Stegbauer, 1974). The reaction mixture consisted of 1% (w/v) starch solution, phosphate buffer (pH 7) and the tissue homogenate. The reaction mixtures were incubated at 37^0C for 30 minutes. DNS was added after incubation and kept in boiling water bath for 5 minutes. After cooling, the absorbance was measured at 540 nm. Maltose was used as the standard. Amylase activity was expressed as mole of maltose released from starch per minute at 37^0C.

Amylase (units per ml enzyme) = {(mg of maltose release) x (dilution factor)} / Volume of enzyme used in ml

Amylase (units mg protein^{-1}) = (units enzyme ml^{-1}) / (mg protein ml^{-1})

Proximate Analysis of Diets

Proximate composition of the experimental diets was analyzed following the standard methods of AOAC (2005). Briefly, moisture was determined by drying the samples at 105^{O}C to a constant weight. Nitrogen content of the samples was measured by Kjeltec (2200 Kjeltec auto distillation, Foss Tecator, Sweden) and crude protein (CP) was calculated by multiplying nitrogen percentage by 6.25. Ether extract (EE) was measured by Soxtec (1045 Soxtec Extraction Unit, Tecator, Sweden) using diethyl ether (boiling point, 40–60^{O}C) as a solvent and ash content was measured by incinerating the samples in a muffle furnace at 600^{O}C for 6 h. Total carbohydrate was calculated by difference, i.e. total carbohydrate% = 100-(CP% + EE% +Ash%).

Statistical Analysis

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 (DMRT) using SPSS (Version 16.0). Results are reported as mean ± S.E. Each tank was considered as an experimental unit for calculating growth, SGR, survival, but for all other parameters triplicate measurements from each tank were done totaling n = 9 per treatment. The level of significance employed was 0.05.

Result

The proximate composition (% dry matter basis) of the experimental diets is presented in Table 1. The crude protein level in the experimental diets was found to be near the formulated value (50%). All the nutrients except fibre content did not have any significant difference ($P>0.05$) among the experimental diets. The fibre content in the control diet is higher than diets supplemented with attractants. The range values of different water quality parameters in different treatments are presented in the Table 2. There was no abrupt change in any parameters of the aquarium water during the whole duration of the experiment.

Supplementation of different chemo attractants significantly ($P<0.05$) affected the growth performance of *Ompok bimaculatus* fry which is presented in table 3. The highest growth was observed in the treatment fed with betaine supplemented diet followed by inosine monophosphate whereas, no significant difference ($P>0.05$) was observed among control, DL-alanine and L-tryptophan supplementation. Other yield parameters such as survival, total biomass, condition factor and performance index are shown in the table 4. The highest survival was found in L-tryptophan supplemented diet (48.66±2.4%) followed by betaine, DL-alanine, control and lowest was found in inosine

monophosphate treatment (32.00±2.0). It has also observed that there was significant difference (P>0.05) in survival between tryptophan and betaine supplemented diets fed groups. The digestive enzyme activity are presented in the table 5. During rearing period, significantly highest (P<0.05) protease enzyme activity was observed in control and betaine supplemented group i.e. 0.086±0.002 and 0.084±0.003 respectively. Higher protease enzyme activity was observed in control and betaine supplemented groups whereas lower activity was found in Dl-alanine and inosine monophosphate supplemented groups. In case of lipase, there is no significant difference among treatments. During experiment, significantly highest (P<0.05) amylase enzyme activity was observed in control (0.042±0.001) followed by betaine supplemented group, followed by alanine supplementation while there was no significant difference between tryptophan and inosine monophosphate.

Discussion

The present study demonstrated that the addition of betaine and inosine monophosphate as chemo-attractants improved the growth performance of pabda fry. This may be due to increased palatability of feed consequently increasing appetite through activation of the cephalic reflex induced by smell and taste of attractive substances in diets (Fange & Grove, 1979, Kasumyan & Doving, 2003) as betaine is a highly water soluble and diffusible compound which has the ability to stimulate the olfactory bulb of fish (Ajiboye, Yakubu, & Adams, 2012). Several other studies also reported that betaine increased the growth in fish such as coho salmon, *Oncorhynchus kisutch* (Castro, Battaglia, & Virtanen, 1998); winter flounders and rainbow trout, *Oncorhynchus mykiss* (Beklevik &

Table 2. Physico-chemical parameters of water in different experimental groups

Parameter	Control	Betaine	DL-alanine	L-tryptophan	Inosine mono phosphate
Dissolve oxygen(mg l^{-1})	8.56- 10.4	8.3-9.9	8.24-8.51	8.1-8.59	8.2-10.4
Temperature(^{o} C)	26.6-28.7	26.6-27.6	26-28	26.5-28.1	26-27.6
Water pH	7.6-7.83	7.6-7.8	7.6-7.87	7.68-7.84	7.42-7.8
Carbon dioxide(mg l^{-1})	2-3	2-3.2	3-4	2.3-3	2.1-3.1
Total alkalinity(mg l^{-1})	50-59.2	52-60	50-58	50-60.4	51-59
Hardness(mg l^{-1})	31.4-40	33-38	34-40	34-39	32-37
Ammonia(mg l^{-1})	0.01-0.03	0.02-0.04	0.02-0.036	0.01-0.03	0.01-0.03

Table 3. Growth parameters (mean±S.E) in *Ompok bimaculatus* fry fed with different chemo-attractants

Parameter	Control	Betaine	DL-Alanine	Tryptophan	Inosine mono phosphate (IMP)
Final length(cm)	2.8±0.02^{a}	2.7±0.02^{a}	2.6±0.01^{a}	2.8±0.11^{a}	3.1±0.05^{b}
Final weight(g)	0.11±0.001^{a}	0.16±0.0008^{c}	0.11±0.0008^{a}	0.11±0.001^{a}	0.12±0.001^{b}
Weight gain(g)	0.03±0.001^{a}	0.08±0.0008^{c}	0.03±0.0008^{a}	0.03±0.001^{a}	0.04±0.001^{b}
Body weight gain (%)	52.90±1.5^{a}	118.3±1.1^{c}	53.3±1.1^{a}	52.90±1.5^{a}	66.4±1.5^{b}
Length increment (cm)	0.49±0.02^{a}	0.42±0.02^{a}	0.37±0.01^{a}	0.53±0.11^{a}	0.84±0.05^{b}
Specific growth rate (% d^{-1})	6.5±0.01^{a}	6.8±0.005^{c}	6.5±0.007^{a}	6.5±0.01^{a}	6.5±0.009^{b}
Mean daily weight gain (%)	0.13±0.003^{a}	0.29±0.002^{c}	0.13±0.002^{a}	0.13±0.003^{a}	0.16±0.003^{b}

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

Table 4. Yield parameters (mean ±S.E) of *Ompok bimaculatus* fry fed with different chemo-attractants

Parameter	Control	Betaine	DL-Alanine	Tryptophan	IMP
Survival (%)	33.33±3.5^{a}	41.33±0.66^{b}	38.00±2.3^{ab}	48.66±2.4^{c}	32.00±2.0^{a}
Total biomass (g)	1.88±0.21^{a}	3.33±0.06^{c}	2.15±0.14^{a}	2.75±0.14^{b}	1.96±0.12^{a}
Condition factor (K)	0.31±0.39^{a}	0.44±0.4^{c}	0.30±0.1^{a}	0.32±1.6^{a}	0.38±0.68^{b}
Performance index	0.043±0.005^{a}	0.120±0.002^{c}	0.050±0.004^{ab}	0.063±0.004^{b}	0.052±0.003^{ab}

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

Table 5. Mean digestive activity in *Ompok bimaculatus* fry reared in aquarium fed with different chemo-attractants

Parameter	Control	Betaine	DL-Alanine	Tryptophan	IMP
Protease (units mg protein^{-1} min^{-1})	0.086±0.002^{c}	0.084±0.003^{c}	0.023±0.0008^{ab}	0.030±0.0008^{b}	0.014±0.005^{a}
Lipase (units mg protein^{-1})	0.135±0.0008	0.168±0.033	0.146±0.036	0.232±0.056	0.189±0.038
Amylase (units mg protein^{-1})	0.042±0.001^{d}	0.028±0.0005^{c}	0.014±0.0003^{b}	0.01±0.0001^{a}	0.009±0.0001^{a}

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

Polat, 2001). In contrast, few studies have reported that betaine did not have any benefecial effect on the growth performance of fishes like *Orechromis aureus* (Genc, Tekelioglu, Yilmaz, Hunt, & Yanar, 2006), *Coregonus schinzi* (Dabrowski & Kaushik 1985), atlantic salmon, *Salmon salar* (Duston, 1993), sea bass, *Dicentrarchus labrax*, and *Leporinus macrocephalus* (Edivaldo, Rodrigo, Robson, & Helton, 2006). Similarly, the increased growth by ionosine monophosphate (IMP) supplementation may be due to the fact that dietary nucleotides help in differentiation of the developing gastro intestinal tract in fish larvae (Uauy, Stringel, Thomas, & Quan, 1990). It is also to be mentioned here that exogenous supply of IMP in di*et al*so promotes the growth of fish and crustaceans in early stages meeting their high rate of cell replication (Borda, Martinez-Puig, & Cordoba, 2003). However, a study with Olive Flounder (*Paralichthys salmoides*) failed to show any positive influence of IMP supplementation on feed intake (Song, Lim, & Lee, 2012). In the same way, Changan, Qiyou, Yaping, Hong, and Sheng (2012) has also reported that supplementation of sodium-5-inosinate in diets did not improve food intake, growth performances, body composition of *Hucho taimen*. The study showed that supplementation of L-tryptophan (TRP) did not show any significant effect (P>0.05) in growth performance of pabda seed. The finding matches with the study of Hseu *et al*., (2003) which showed that the fish fed with TRP expressed lower growth rates and suggested that this could be due to increased brain serotonergic activity and decreased aggression and/or appetite. Papoutsoglou, Karakatsouli, and Chiras (2005) also reported that tryptophan (2%) supplemented diets resulted in depressed growth in rainbow trout. Similar observations have been found by Papoutsoglou and Koustas (2005) in *Dicentrarchus labrax*. It is well established that the dietary supplementation of TRP raises brain 5-HT concentration that in turn significantly reduces feed intake (Pinchasov, Fancher, Burke, & Jensen, 1989; De Pedro, Pinillos, Valenciano, Alonso-Bedate, & Delgado, 1998). However, Tejpal *et al*., (2009) has found that supplementation of L-Tryptophan mitigates the crowding stress in *Cirrhinus mrigala* fingerlings and enhances the growth, SGR and feed efficiency. Few studies have described that TRP supplementation did not have any stimulatory effect on feed intake (Lepage, Tottmar, & Winberg, 2002 ; Winberg, Øverli, & Lepage, 2001). It has been reported by Yilmaz (2005) that DL-alanine did not improve the growth and survival of *Clarias gariepinus* larvae, which supports the findings of the present study.

In the present study, fish fed with L-tryptophan supplemented diet showed the significantly highest (P<0.05) survival followed by betaine supplementation, whereas supplementation of alanine and inosine monophosphate showed no significant difference. Aggression is one of the important behavior in carnivorous fishes like Pabda and feed supplemented with tryptophan can suppress this behavior and similar studies have been found viz. *Oncorhynchus mykiss* (Winberg, *et al*., 2001), Amazonian characid, *Brycon amazonicus* (Wolkers, Serra, Hoshiba, & Urbinati, 2012), Rainbow trout (Overli, Harris, & Winberg, 1999 and Lepage, *et al*., (2002), Atlantic cod (Hoglund, Bakke, Øverli, Winberg, & Nilsson, 2005). Hseu *et al*., 2003 found that dietary supplementation of L- tryptophan in juveniles rainbow trout diet leads to inhibit aggression behavior. Hoglund, Sorensen, Bakke, Nilsson, & Øverli, 2007 also revealed that L-tryptophan reduce cannibalism and stress induced anorexia in juvenile grouper and Lepage, Vilehez, Pottinger and Winberg, (2003) showed that it prevents stress induced cortisol surge. In contrast, Krol, Flisiak, Urbanowicz, and Ulikowski, (2014) reported that the tryptophan supplementation had no significant effect on growth or survival of *Silurus glanis.*

The study showed that total biomass and performance index is significantly higher (P<0.05) in betaine supplementation followed by tryptophan supplementation. This may be due to more individual growth in case of betaine and more survivability in case of TRP supplementation. Condition factor was significantly higher (P<0.05) in betaine supplementation followed by L-tryptophan because of the better palatability which offered better food intake consequently gave improved condition factor.

The result of the current study suggested that betaine fed group showed higher digestive enzyme activity than the other supplemental group. The higher digestive enzyme activity contributes to more efficient digestion which reflected in the higher growth of the fishes. The mechanism in which betaine

stimulates the activity of digestive enzyme has not been investigated in fish yet. However, Eklund, Bauer, Wamatu, and Mosenthin, 2005 reported that betaine supports intestinal growth and function consequently contributing to the enhancement of digestive enzyme activity. The significantly ($P<0.05$) higher protease have been found in control group which may be due to the lower feed intake which in turn increases the capacity of protein digestion. In the current study, the control diet provoked canabalism, thereby consumption of own siblings which also may be contributed to more protease activity. Similar result also reported by Bolasina, Perez, and Yamashita, (2006). In our study, lower protease, amylase and lipase activity have been found in tryptophan supplemented group which might be due to suppressive effect of TRP on digestive system resulting lower enzymatic function. The lipase content mainly depend on nature of food, developmental stage and the species (Sharma & Chakrabarti, 1999) which were kept same in different treatment, which resulted no significant difference among the treatment. The amylase activity is directly related to the amount of dietary carbohydrate. Since, the carbohydrate source cellulose has been replaced with the chemo attractants, the higher amylase activity in control fed group was observed than the control group. In case of control and alanine supplemented diets, the availability of nutrients for digestion drops down as feed intake declines which can be partly due to unpleasant palatability of the diets.

Conclusion

From the foregoing discussion, it can be concluded that the dietary addition of betaine and inosine mono phosophate at 2% level can give improved growth performance during pabda fry rearing in controlled condition. Most interestingly, dietary tryptophan supplementation could be a promising aquaculture management strategy for carnivorous fish as it showed significantly better survival without affecting the growth. Moreover, feed supplemented with tryptophan induce decreased size heterogeneity in pabda which attributed to the survival of young ones thereby suggesting further study of optimal time (earlier, e.g., at co-feeding procedure) or optimal dose of tryptophan inclusion. As the performance of a hatchery mainly judged based on the survivor rather than the growth, the supplementation of L-tryptophan can be considered to be the best. The current study is totally laboratory based, so further fields studies are needed to clarify the actual effect of these feed attractants on the fish with its best level of addition.

Acknowledgment

The authors are thankful to the Prof. Premjit Singh, Vice Chancellor, Central Agricultural University, Imphal and Dr. Pramod Kumar Pandey, Dean, College of Fisheries, CAU (I), Lembucherra, Tripura, for providing the necessary research facilities.

References

Ajiboye, O. O., Yakubu, A. F., & Adams, T. E. (2012). A perspective on the ingestion and nutritional effects of feed additives in farmed fish species. *World Journal of Fisheries and Marine Sciences*, *4*(1), 87-101. http://dx.doi.org/10.5829/idosi.wjfms.2012.04.01.56264

AOAC. (2005). *Association of Official Analytical Chemists, Official Methods of Analysis*. 18th Edition. Rev. 2, 2007. Gaithersburg, MA

APHA, AWWA, & WEF. (1998). *Standard methods for examination of water and wastewater*, 20th Edition. American Public Wealth Association, Washington, DC, USA

Banik, S., Goswami, P., & Malla, S. (2012). Studies on breeding physiology of *Ompok bimaculatus* (Bloch, 1794) in Tripura. *Uttar Pradesh Journal of Zoology*, *32*(1), 67-72.

Baras, E., & Jobling, M. (2002). Dynamics of intracohort cannibalism in cultured fish. *Aquaculture research*, *33*(7), 461-479. http://dx.doi.org/10.1046/j.1365-2109.2002.00732.x

Beklevik, G., & Polat, A. (2001). Effects of DL alanine and betaine supplemented diets on the growth and body composition of fingerling rainbow trout (*Oncorhynchus mykiss*, W. 1972). *Turkish Journal of Veterinary and Animal Sciences*, *25*, 301-307.

Bloch, M. E. (1794). Naturgeschichte der ausländischen Fische (*Natural history of foreign fish*). Schlesinger, Berlin. *8*, 174.

Bolasina, S., Perez, A., & Yamashita, Y. (2006). Digestive enzymes activity during ontogenetic development and effect of starvation in Japanese flounder, *Paralichthys olivaceus*. *Aquaculture*, *252*(2), 503-515. http://dx.doi.org/10.1016/j.aquaculture.2005.07.015.

Borda, E., Martinez-Puig, D., & Cordoba, X., (2003). A balanced nucleotide supply makes sense. *Feed Mix*. *11*, 24-26.

Castro, H., Battaglia, J., & Virtanen, E. (1998). Effects of FinnStim on growth and sea water adaptation of Coho salmon. *Aquaculture*, *168*(1), 423-429. http://dx.doi.org/10.1016/S0044-8486(98)00368-8

Changan, W., Qiyou, X. U.,Yaping, C., Hong, X. U., & Sheng, J. Y. (2012). Effects of feeding attractants on growth and body composition, digestive enzyme and serum indices of *Hucho taimen*. *Animal Husbandry and Feed Science*, *4*(5), 236-240.

Cherry, I. S., & Crandall, L. A. (1932). The specificity of pancreatic lipase: its appearance in the blood after pancreatic injury. *American Journal of Physiology-Legacy Content*, *100*(2), 266-273.

Dabrowski, K.R., & Kaushik, S.J. (1985). Rearing of coregonid (*Coreconus schinzi pallea* Cuv. et Val.) larvae using dry and live food. III. Growth of fish and developmental characteristics related to nutrition. *Aquaculture*, *48*, 123–135. http://dx.doi.org/10.1016/0044-8486(85)90099-7

De Oliveira, A., & Cyrino, J. (2004). Attractants in plant protein-based diets for the carnivorous largemouth bass *Micropterus salmoides*. *Scientia Agricola*, 61,

326-331.

De Pedro, N., Pinillos, M.L., Valenciano, A.I., Alonso-Bedate, M., Delgado, M.J. (1998). Inhibitory effect of serotonin on feeding behaviour in goldfish: involvement of CRF. Peptides *19*(3), 505–511. http://dx.doi.org/10.1016/S0196-9781(97)00469-5

Drapeau, G. (1974). Protease from *Staphylococcus aureus*. In B. L. Lorand (ed.), *Methods in Enzymology*, academic press, New York, USA, *45B*, 469.

Duston, J. (1993). Effects of dietary betaine and sodium chloride on seawater adaptation in Atlatic salmon parr (*Salmo salar* L.). *Comparative Biochemistry and Physiology Part A: Physiology*, *105*(4), 673-677. http://dx.doi.org/10.1016/0300-9629(93)90267-8.

Edivaldo, B. N., Rodrigo, E. B., Robson, F. C., & Helton, C. D., (2006). Effects of Betaine on the Growth of the Fish Piauçu, *Leporinus macrocephalus*. *Brazilian Archives of Biology and Technology*. *49*, 757-762.

Eklund, M., Bauer, E., Wamatu, J., & Mosenthin, R. (2005). Potential nutritional and physiological functions of betaine in livestock. *Nutrition research reviews*, *18*(01), 31-48. http://dx.doi.org/10.1079/NRR200493

Fange, R., & Grove, D. S. (1979). Digestion, in W. S. Hoar. D. J., Randall & J. R. Brett (eds.), *Fish Physiology*, *Vol. 8*. (pp. 162-260). Acad. Press, NY.

Gaber, M. (2005). The effect of different levels of krill meal supplementation of soybean-based diets on feed intake, digestibility, and chemical composition of juvenile Nile Tilapia *Oreochromis niloticus*, L. *Journal of the World Aquaculture Society*, *36*(3), 346-353. http://dx.doi.org/10.1111/j.1749-7345.2005.tb00338.x

Genc, M.A., Tekelioglu, N., Yilmaz, E., Hunt A.O., & Yanar, Y. (2006). Effect of dietary betaine on growth performance and body composition of *Oreochromis aureus* reared in fresh and dead water comparative study. *Journal of Animal and Veterinary Advances*, *5*(12): 1185-1188.

Hecht, T., & Pienaar, A. G. (1993). A review of cannibalism and its implications in fish larviculture. *Journal of the World Aquaculture Society*, *24*(2), 246-261. http://dx.doi.org/10.1111/j.1749-7345.1993.tb00014.x

Hoglund, E., Bakke, M. J., Øverli, Ø.,Winberg, S., & Nilsson, G. E., (2005). Suppression of aggressive behaviour in juvenile Atlantic cod (*Gadus morhua*) by L-tryptophan supplementation. *Aquaculture*, *249*(1), 525-531. http://dx.doi.org/10.1016/j.aquaculture.2005.04.028

Hoglund, E., Sorensen, C., Bakke, M. J., Nilsson, G. E., and Øverli, Ø. (2007). Attenuation of stress-induced anorexia in brown trout (*Salmo trutta*) by pre-treatment with dietary L-tryptophan. *British Journal of Nutrition*, *97*, 786–789. http://dx.doi.org/10.1017/S0007114507450280.

Hseu, J. R., Lu, F. I., Su, H. M., Wang, L. S., Tsai, C. L., & Hwang, P. P. (2003). Effect of exogenous tryptophan on cannibalism, survival and growth in juvenile grouper, *Epinephelus coioides*. *Aquaculture*, *218*(1), 251-263. http://dx.doi.org/10.1016/S0044-8486(02)00503-3

Hughes, S. G. (1991). Response of first feeding spring Chinook Salmon to four potential chemical modifiers of feed intake. *The Progressive Fish Culturist*, *53*, 17-20. http://dx.doi.org/10.1577/1548-8640(1991)053<0015:CROFFS>2.3.CO;2

Ishida, Y., & Hidaka, I. (1987). Gustatory response profiles for amino acids, glycine betaine, and nucleotides in several marine teleosts. *Nippon Suisan Gakkaishi*, *53*(8), 1391–1398. http://dx.doi.org/10.2331/suisan.53.1391

Jayasankar, P. (2012). Recent advances in freshwater finfish aquaculture - Prospects and constraints. In: K. Das Mahapatra, P. Routray, N. K. Barik, P. Jayasankar (eds.). Quality fish seed production through brood fish management in SAARC countries. CIFA, Bhubaneswar, India. 14-21 pp.

Kasumyan, A. O., & DÖving, K. B. (2003). Taste preferences in fishes. *Fish and fisheries*, *4*(4), 289-347. http://dx.doi.org/10.1046/j.1467-2979.2003.00121.x

Kiyohara, S., Hidaka, I., & Tamura, T. (1975). Gustatory response in the Puffer-II. Single fiber analyses. *Bulletin of the Japanese Society of Scientific Fisheries*, *41*(4), 383-391. http://dx.doi.org/10.2331/suisan.41.383.

Kolkovski, S., Arieli, A., & Tandler, A. (1997). Visual and chemical cues stimulate microdiet ingestion in sea bream larvae. *Aquaculture International*, *5*(6), 527-536. http://dx.doi.org/10.1023/A:1018305416501.

Krol, J., Flisiak, W., Urbanowicz, P., & Ulikowski, D. (2014). Growth, cannibalism, and survival relations in larvae of European catfish, *Silurus glanis* (Actinopterygii: Siluriformes: Siluridae)—attempts to mitigate sibling cannibalism. *Acta Ichthyologica Et Piscatoria*, *44*(3), 191–199.

Lepage, O., Tottmar, O., & Winberg, S. (2002). Elevated dietary intake of L-tryptophan counteracts the stress-induced elevation of plasma cortisol in rainbow trout (*Oncorhynchus mykiss*). *Journal of Experimental Biology*, *205*(23), 3679-3687.

Lepage, O., Vilehez, I. M., Pottinger, T. G., & Winberg, S., (2003). Time-course of the effect of dietary L-tryptophan on plasma cortisol levels in rainbow trout *Oncorhynchus mykiss*. *The Journal of Experimental Biology*, *206*, 3589-3599. http://dx.doi.org/10.1242/jeb.00614

Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951): The method for protein estimation. *Journal of Biological Chemistry*, *193*, 265-275

Mackie, A.M., & Adron, J.W. 1978. Identification of inosine and inosine 5-monophosphate as the gustatory feeding stimulants for the turbot, *Scophthalmus maximus*. *Comperative Biochemistry and Physiology*, *60*A, 79–83. http://dx.doi.org/10.1016/0300-9629(78)90040-3.

Ng, H. H., Tenzin, K., & Pal, M. (2010). *Ompok bimaculatus*. The IUCN Red List of Threatened Species.Version 2015.1.

Overli, O., Harris, C. A., & Winberg, S. (1999). Short-term effects of fights for social dominance and the establishment of dominant-subordinate relationships on brain monoamines and cortisol in rainbow trout. *Brain, Behavior and Evolution*, *54*(5), 263-275. http://dx.doi.org/10.1159/000006627.

Papatryphon, E., & Soares, J. H. (2001). Optimizing the levels of feeding stimulants for use in high fish meal and plant feedstuff based diets for striped bass, *Morone saxitilis*. *Aquaculture 202*(3), 279-288. http://dx.doi.org/10.1016/S0044-8486(01)00778-5

Papoutsoglou, N. K., & Koustas, P. (2005). Effects of dietary L- tryptophan and lighting conditions on growth performance of European sea bass,

Dicentrarchus labrax (Linnaeus) juveniles reared in a recirculating water system. *Journal of Applied Ichthyology*, *21*(1), 520-524. http://dx.doi.org/10.1111/j.1439-0426.2005.00664.x

Papoutsoglou, S. E., Karakatsouli, N., & Chiras, G. (2005). Dietary L-tryptophan and tank colour effects on growth performance of rainbow trout (*Oncorhynchus mykiss*) juveniles reared in a recirculating water system. *Aquacultural engineering*, *32*(2), 277-284. doi: 10.1016/j.aquaeng.2004.04.004

Pinchasov, Y., Fancher, B., Burke, W. H., & Jensen, L.S. (1989). Clycolic acid and tryptophan effects on feed intake and hypothalamic indoleamines in chicks. *Physiology & Behavior*. *45*, 585-589. http://dx.doi.org/10.1016/0031-9384(89)90077-2

Rick, W. and Stegbauer, H. P., 1974. Amylase measurement of reducing groups, In H. V. Bergmeyer (ed.), *Methods of Enzymatic Analysis*, 2nd ed., Academic Press, New York, *2*: 885-889.

Shankar, R., Murthy, H. S., Pavadi, P., & Thanuja, K. (2008). Effect of betaine as a feed attractant on growth, survival, and feed utilization in fingerlings of the Indian major carp, *Labeo rohita*. *Israeli Journal of Aquaculture-Bamidgeh*. *60*, 95–99. http://hdl.handle.net/10524/19250

Sharma J. G., & Chakrabarti R. (1999). Larval rearing of common carp *Cyprinus carpio*: A comparison between natural and artificial diets under three stocking densities. *Journal of the world aquaculture society*, *30*(4), 490-495. http://dx.doi.org/10.1111/j.1749-7345.1999.tb00997.x

Smith, D. M., Tabrett, S. J., Barclay, M. C., & Irvin, S. J. (2005). The efficacy of ingredients included in shrimp feeds to stimulate intake. *Aquaculture Nutrition*, *11*, 263−271, http://dx.doi.org/10.1111/j.1365-2095.2005.00349.x

Sola, C., & Tongiorgi, P. (1998). Behavioral responses of glass eels of *Anguilla anguilla* to non-protein amino acids. *Journal of Fish Biology*, *53*(6), 1253-1262. http://dx.doi.org/10.1111/j.1095-8649.1998.tb00246.x

Song, J. W., Lim, S. J., & Lee, K. J. (2012). Effects of dietary supplementation of inosine monophosphate on growth performance, innate immunity and disease resistance of olive flounder (*Paralichthys olivaceus*). *Fish Shellfish Immunology*, *33*, 1050–1054. http://dx.doi.org/10.1016/j.fsi.2012.07.011

Tandler, A., Berg, B. A., & Mackie, A. M. (1982). Effect of food attractants on appetite and growth rate of gilthead bream, *Sparus aurata* L. *Journal of Fish Biology*, *20*(6), 673-681. http://dx.doi.org/10.1111/j.1095-8649.1982.tb03977.x

Tejpal, C. S., Pal, A. K., Sahu, N. P., Kumar, J. A., Muthappa, N. A., Vidya, S., & Rajan, M. G. (2009). Dietary supplementation of L-tryptophan mitigates crowding stress and augments the growth in *Cirrhinus mrigala* fingerlings. *Aquaculture*, *293*(3), 272-277. http://dx.doi.org/10.1016/j.aquaculture.2008.09.014

Uauy, R., Stringel, G., Thomas, R., & Quan, R. (1990). Effect of dietary nucleotides on growth and maturation of the developing gut in the rat. *Journal of Pediatric Gastroenterology and Nutrition*, *10*, 497–503.

Winberg, S., Øverli, Ø., & Lepage, O. (2001). Suppression of aggression in rainbow trout (*Oncorhynchus mykiss*) by dietary L-tryptophan. *Journal of Experimental Biology*, *204*(22), 3867-3876.

Wolkers, C. P. B., Serra, M., Hoshiba, M. A., & Urbinati, E. C. (2012). Dietary L-tryptophan alters aggression in juvenile matrinxa *Brycon amazonicus*. *Fish physiology and biochemistry*, *38*(3), 819-827. http://dx.doi.org/10.1007/s10695-011-9569-x.

Yilmaz, E. (2005). The effects of two chemo-attractants and different first feeds on the growth performances of African catfish (*Clarias gariepinus*, Burchell, 1822) at different larval stages. *Turkish Journal of Veterinary Animal Science, 29*, 309-314.