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RESEARCH ARTICLE

PROBIOTIC ENRICH DIETARY EFFECT ON THE REPRODUCTION OF BUTTER CATFISH, *OMPOK PABDA* (HAMILTON, 1872)

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ABSTRACT

A study was directed to assess probiotic-enrich dietary effect on the reproductive performance and larvae growth produced from the brood fish of *Ompok pabda* for 60 days. A total of 120 pairs of broodfish was used and fed with diets incorporating different levels of probiotic viz. 0 (served as a control), 0.6, 0.8 and 1.0g / kg diet under 4 treatments, i.e. PRO0, PRO1, PRO2 and PRO3 having 3 replications of each. After feeding trials, the brood fish were induced to breed with equal doses of ovaprim i.e. 0.5 and 1.2ml/kg body weight for male and female, respectively and subsequently larvae were produced. The experimental fish were observed for the success in reproductive performance like Gonado Somatic Index (GSI), fecundity, percentage of fertilization, percentage of hatching, percentage of deformed and formed larvae. GSI (10.24±0.94), fertilization rate (76.07±1.67) and percentage of hatching (78.65±4.17) found increased in PRO2 treatment. Likewise, in case of growth rate PRO2 also showed the best results compared to other treatments. Besides these, percentage of dead (21.71±2.6) and deformed larvae (1.85±1.5) were significantly lower in fish fed with the probiotic (0.8g) feed. Interestingly, PRO3 treatment, in some cases, gave the lowest result, in contrast to other treatments. So, it can be said that the use of higher concentration of the probiotic in diet did not always lead to significantly improved reproductive performance of spawners. Overall, the results demonstrated the beneficial effects of probiotics on the reproductive performance of this indigenous species, as the GSI, fecundity and larval survival were significantly enhanced by probiotic administration. So, it could be concluded that the probiotic is useful for enhancing fish growth, reproduction efficiency (especially for females) and development of fish gonad. So, probiotic can be used as broodstock feed additive in hatcheries which have a greater economic point of view.

Key words: Gonado Somatic Index (GSI), Fecundity, Fertilization and Hatching.

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INTRODUCTION

The *Ompok pabda*, territorially known as Pabda, a small freshwater catfish pertaining to the family Siluridae of the order Siluriformes (Siddiqua *et al.,* 2000). This fish species is considered as palatable and alimentary to the people of East India, North East India and Bangladesh (Jhingran, 2004). This was plenty in the past, however, recently decreasing their natural stocks due to over-exploitation and various ecological changes. Moreover, *O. pabda* is listed as one of the endangered fish species in Bangladesh (IUCN, 1998). Although its production can be increased through culture practice but it is very difficult to collect sufficient number of fry and fingerlings of this catfish from natural sources for stocking in the ponds and survival rate is low as well. In addition, the fry and juveniles of this species exhibit cannibalistic tendencies (Parameswaran *et al.,* 1971) which creates management problem. The role of nutrition on reproduction are well known and widely recognized. Nutrition affects all the events of reproduction, even from the stage of puberty to gametogenesis, in both sexes (male and female). This relation between nutrition and reproduction proves that reproductive events are closely associated with the nutrient supply (Scaramuzzi *et al.,* 2006) in order to ensure the survival of new progeny. In many endangered teleost species, especially those unsettled to aquaculture, variable reproductive performances are important restrictive factors for the successful mass production of offspring. Slight improvement in broodstock nutrition and alimentation even can greatly

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enhance gamete quality, hatching rate, survival rate and likewise fry production, because essential dietary nutrients affect gonadal development and fecundity (Izquierdo *et al.,* 2001). Recently, major portion of animal protein represents fish aquaculture (Lara-Flores *et al.,* 2003) but culture system especially in intensive fish farming with high stocking densities and high nutrient inputs can facilitate the multiplication of opportunistic bacteria (Austin *et al.,* 1995). The excessive use of antibiotics, drugs, pesticides and antiseptics to prevent bacterial diseases and to promote juvenile growth has led to the development of resistant bacterial strains and subsequent potential safety issues (Akinbowale *et al.,* 2006). Increase in bacterial resistance to antibiotics has made attention to research into the usage of probiotics, as it can exert benefits on host welfare other than nutritional support (Bengmark, 1998). In spite of many prospects, not much attempts have been made to popularize its commercial culture and mass production of fry and its fingerlings stands as one of the major hindrance towards its aquaculture. So, it is needed to increase the gonadal performance for smooth supply of quality fry not only for sustainable aquaculture but also protect this species from extinction using probiotic rich diet. Therefore, the research was conducted to investigate the effects of dietary probiotic administration on the reproductive performances of the freshwater catfish, *O. pabda* and the effects of such broodstock dietary treatment on the growth and survival of new progeny.

MATERIALS AND METHODS

In order to observe the probiotic enrich dietary effects on the reproduction of butter catfish *O. pabda,* present experiment was setup into two phases. At first phase, brood fish were reared & maintained in the tanks for 60 days by providing different levels of probiotic to observe the growth and gonadal development of broodfish and in the second phase, evaluation of breeding performances (ovulation rate of the female, fertilization rate, hatching rates of the eggs, survival and growth rate of the larvae) of the reared broods.

Experimental samples

About 300 *O. pabda* fish samples (breeding season from May to July) were collected from Bangladesh Fisheries Research Institute (BFRI) hatchery, Mymensingh, Bangladesh. After receiving, they were disinfected by immersing in 5 ppm potassium permanganate solution for 15minutes and transferred to circular plastic tanks of 500 L capacity containing filtered tube-well water with permanent aeration and maintained in such condition for 3 months with proper circulation and filtration until they reached sexual maturity. The sex determination of fishes was based on secondary sexual characters. Then healthy, strong and more or less equal sized fishes were used for the experiment.

Probiotic strains

The probiotic bacterial strain, *Lactobacillus rhamnosus*, used in the study was isolated from the intestine of *Labeo rohita* (Rui) (weight 315 g and length 32 cm) and identified based on its morphological and physiological characteristics (Sneath, 1986; Priest, 1993). The strain was selected for its *in vitro* inhibitory property against fish pathogen *Aeromonashydrophila* and *Edwardsiellatarda* in the double layer (Dopazo *et al.,* 1988) technique.

Preparation of probiotic bacterial cell suspension

The probiotic bacterial strain, *Lactobacillus rhamnosus*, was maintained by freezing under -70^{o}C in 10% v/v of glycerol in tryptone soya broth (Merck). Then it was recovered and incubated at 30 ºC for 48 hrs. The cells were harvested by centrifugation at 5000 rpm for 25mins at 4ºC in a cooling centrifuge (C-23 plus, Mumbai, India). The cell pellets were suspended in 50mL sterile normal saline solution (NSS) and used immediately.

Preparation of control and probiotic feed

A mixed diet was prepared with the ingredients including fish meal (40%), rice bran (10%), wheat bran (10%), soybean meal (17.56%), mustard oil cake (15%), and wheat flour (5%) containing 35% protein for treating all experimental fishes. The dough prepared by adding required amount of water to these ingredients was steam sterilized (autoclave at 121 ºC for 15mins) and incorporated with a commercial vitamin-mineral mix (Square Pharmaceuticals Ltd. Bangladesh) at 2% (v/w) and pelletized using a hand pelletizer to obtain 1mm pellets. The pellets were initially sun dried and then dried in an oven at 60 ºC for 12hrs to <10% moisture content. They were manually broken into smaller bits and stored in air-tight sterile polypropylene containers at room temperature.

Table 1. Composition of experimental diets

Proximate composition	Treatments			
	PRO_0	PRO_1	PRO_2	PRO_3
Moisture	8.40	10.54	35.13	14.97
Lipid	8.20	10.12	34.96	15.16
Protein	8.17	10.11	35.20	16.44
Ash	7.56	10.17	35.04	16.23

The probiotic test feeds T_1 (PRO_0, no probiotic as control), T_2 (PRO_1), T_3 (PRO_2) and T_4 (PRO_3) were prepared by gently spraying the required amount of bacterial suspension on the diet and mixing it part by part in a drum mixer to obtain a final probiont concentration of 5×10^6 cells g^{-1} (PRO_1), 5×10^7 cells g^{-1} (PRO_2), and 5×10^8 cells g^{-1} (PRO_3), respectively. The proximate composition (moisture, protein, lipid and ash) of all probiotic feeds and control feed were determined (Table 1) using the standard procedures of AOAC (1990) in the Nutrition Laboratory of Faculty of Fisheries, BSMRAU, Gazipur. The probiotic strain-incorporated feeds were packed in sterile polypropylene containers and stored at 4 ^{0}C for viability studies. The total counts of probiotic bacterium in their respective feeds were determined on the 0th, 30th, 60th day of storage (Table 2) by spread plating on TSA.

Table 2. Log counts g^{-1} of probiotic strain in probiotic incorporated feeds during storage Feed

Experimental diets	0th day of storage	21th day of storage	42th day of storage
PRO_1	6.43 ± 0.23	6.28 ± 0.18	5.59 ± 0.21
PRO_2	7.47 ± 0.13	7.38 ± 0.36	6.88 ± 0.04
PRO_3	8.12 ± 0.23	7.92 ± 0.31	7.66 ± 0.16

Values are mean ± SD for three samples of each feed.

Experimental design

For this study 12 spawning tank was divided into four groups including one control group and three probiotic treatment groups having three replications of each. Each spawning tank

was stocked with males and females by using random sampling. Feed with four different levels of probiotic 0g (PRO_0), 0.6g (PRO_1), 0.8g (PRO_2) and 1.0g (PRO_3) probiotic/kg feed were administered for studying the growth and gonadal development of this fish.

Exposure and rearing conditions

The feeding trial was conducted in the Faculty of Fisheries, BSMRAU, Gazipur, in circular plastic tubs/spawning tanks for 60 days. Before feeding the experimental feed the initial weight of all the fishes was noted. Fishes were divided into four and the groups were fed with different concentrations of 0.6 (PRO_1) and 0.8 (PRO_2) and 1.0 gm. (PRO_3) of probiotic supplemented feeds during the experimental period. The experimental diets were given to the fishes twice a day and the control group was also being maintained. The fish were fed with feed at 5% of their body weight daily into two split doses throughout the experimental period. The unutilized feed and fecal matter were collected before each morning and water was changed one in every two days.

Observation of Physico-chemical condition of water

Temperature, dissolved oxygen (DO) and pH of water in each tank under each treatment were recorded. The temperature was recorded by using Celsius thermometer, DO was measured by a digital DO meter and pH was measured by a portable digital pH meter.

Gonadosomatic index (GSI) assessment

The following formula: $GSI\ (\%) = \frac{\text{Weight of gonad (g)}}{\text{Weight of body(g)}} \times 100$

Fecundity assessment

Gravimetric method was used to estimate the fecundity of fish. This was done by the following formula:

$$F = \frac{N \times \text{Gonad weight}}{\text{Sample weight}}$$

Where, F is the fecundity of fish and N is the number of eggs in the sample.

In order to study the probiotic enrich dietary effect on the growth and ovarian developments following parameters were studied.

i) Weight gain (g) = Mean final weight-mean initial weight.
ii) Specific growth rate:

$$SGR\ (\%\ day) = \frac{\ln W2 - \ln W1}{T2 - T1} \times 100$$ (Brown, 1957)

Where, W_1 = The initial live body weight (g) at time T_1 (day).
W_2 = The final live body weight (g) at time T_2 (day).

Ova diameter

At different stages of the maturity the diameter of the ovum was measured with an ocular micrometer under stereoscopic Olympus microscope along the longest axis of the ovum.

Effect of probiotic on the breeding performances

After rearing for two months broodfishes of 3 replicates of each treatment were kept in one tank. Then 6 females from each treatment were selected to perform induced breeding to determine the effect of probiotic on the breeding performance. So, 24 broodfishes from four treatments were kept in 4 different tanks for about 6 hours for conditioning prior to injection.

Inducing agent and preparation of injection

For inducing ovulation of females, the commercially marketed ovaprim hormone was used. Generally ovaprim was applied at 1 – 1.5 ml/kg body weight for females and 0.5-1.0 ml/kg body weight for males, applied in a single injection (Chakrabarti *et al.,* 2009).

Injecting broodfish

During the administration of injection fish were wrapped in a soft and wet cloth and kept lying on soaked foam. The ovaprim solutions were injected intramuscularly on dorsal region behind pectoral fin of the broodfish for stimulation. The needle pushed about 45^0 angle to the body surface during the time of injection.

Collection of eggs and sperms

After injection of ovaprim hormone the females were checked for ovulation after 10-12 hours of injection. As soon as the female fish ovulated the eggs were collected by stripping the fish. Milt was obtained from males by surgically removing the testes, which were macerated. To ensure fertilization the sperm suspension was mixed with eggs by gently stirring with a feather and then a little water was added to the egg-sperm mixture to activate sperms to fertilize the eggs.

Embryo fertilization rate and hatching rate assessment

For calculation of fertilization and hatching rate of eggs produced by the females of each treatment a portion of eggs was taken and incubated in 3 separate bowls (2 liters). The remaining eggs of the females under each treatment were incubated in separate bowls. All the incubation bowls were provided with gentle showers having water flow from porous PVC pipe and outlet facility to ensure adequate oxygen. Soon after fertilization the embryonic development started and the fertilized eggs look blackish or transparent while unfertilized eggs looked whitish. The fertilization rate for the respective treatment was then calculated by using the formula.

$$\text{Fertilization rate (\%)} = \frac{\text{No.of fertilized eggs} \times 100}{\text{Total no.of eggs (fertilized + unfertilized)}}$$

And the hatching rate (calculated as the percentage of the number of viable larvae after hatching, divided by the number of fertilized eggs) was monitored until hatching occurred. The rate of hatching respective treatment was then calculated by using the formula:

$$\text{Hatching rate (\%)} = \frac{\text{No.of eggs hatched} \times 100}{\text{Total no.of eggs}}$$

Survival rate assessment

The survival rate of respective treatment was calculated by using the formula:

$$\text{Survival rate (\%)} = \frac{\text{No.of larvae harvested} \times 100}{\text{No.of larvae stocked}}$$

Progeny biometric parameter assessment

To assess the potential beneficial health effects of broodstock probiotic-based diet, 50 larvae hatched from each experimental group was reared in 5 L tanks for a period of 30 days. Every group was maintained in the same rearing condition as the broodstocks and was fed with *Artemiasalina* nauplii. Larval survival was monitored during the experiment and larval biometric parameters, such as BW (mg) and TL (mm), was recorded during sampling at day 7 days (start), 14, 21 and 28 days (end).

RESULTS

Growth performances of *O. pabda*

Probiotic compound is referred to as a non-digestible feed ingredient that beneficially affects the host by stimulating the growth and improving its intestinal balance. The gradual increases of the probiotic levels led to significant ($P \leq 0.05$) increases (proportional to the increase of probiotic level) in the final body weight, total weight gain (TWG) and specific growth rate (SGR). The data regarding the growth of *O. pabda* fed with three different concentrations of probiotic incorporated diets during the experimental period is presented in Table 3. The average initial weights in four treatments were 25.23±0.6, 25.37±0.37, 24.51±1.0, 23.77±1.89. At the end of the 60 days experimental period, the final weight of brood fish of four treatments were 32.49±0.60, 37.69±1.13, 41.27±2.30, and 34.28±1.93, respectively in treatments PRO_0, PRO_1, PRO_2 and PRO_3. Probiotic feed PRO_2 fed fish showed the higher weight gain (16.76±2.76) followed by PRO_1 (12.32±1.0), PRO_3 (10.95±2.53). A significant ($P<0.05$) decline in the average weight gain (7.26±1.20) was observed in fish fed the control feed (PRO_0). Moreover, specific growth rate (% day) of *O. pabda* brood fish fed on feeds containing different levels of probiotic showed a similar trend to that of weight increase (Table 1). There was a significant difference ($P<0.05$) among four treatments. However, maximum feed conversion ratio (FCR) found in PRO_0 treatment and minimum was in PRO_3treatment where vice-versa results were found for Protein Efficiency Ratio (PER) (Table 3).

Reproductive performances of *O. pabda*

Ganado-Somatic index

Gonado-somatic index give a clear indication about the gonadal development as well as breeding season of a fish. Data of gonado-somatic index is presented in Figure 1. In this case highest gonado-somatic index was found in PRO_2 (10.24±0.94) followed by PRO_1 (10.18±0.78), PRO_3 (9.94±0.77) and PRO_0 (9.62±1.71). The ANOVA test showed that there was a significant difference among treatments regarding gonado-somatic index.

Fecundity: The average fecundity in *O. Pabda* increased with an increase in the concentration of probiotic supplementation in the feed where the probiotic feed PRO_1 fed fish exhibited the maximum average fecundity (1408.98±120.95) per female (Table 4). Although there was no significant difference among three probiotic treatmentsP RO_2 (1376.4±193), PRO_3 (1275.06±263.34) but they showed increased results compared to non-probiotic treatment PRO_0 (1138.55±152.42).

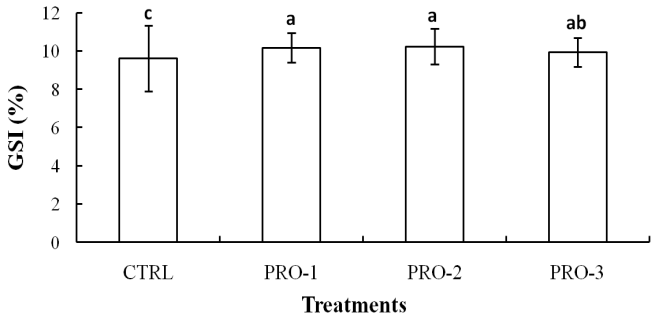

Fig. 1. Comparison of gonado-somatic index of O. *pabdar* eared under different levels of probiotic

Fertilization rate: Fertilization rate of *O. pabda* eggs were greatly influenced by probiotic feed although different probiotic concentration had no significant effect on it (Figure 2). Highest fertilization rate (76.07±1.67) was recorded in PRO_2 followed byPRO_1 (73.86±1.44) and PRO_3 (72.17±5.675) compared to the lowest fertilization rate in PRO_0 (59.24±2.853).

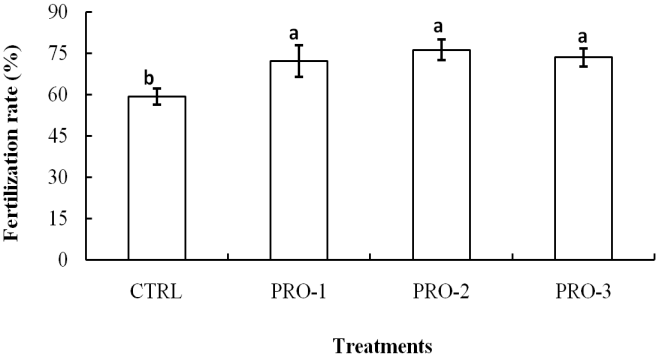

Fig. 2. Comparison of fertilization rate of the eggs produced by the O. *pabda* Brood fish reared under different levels of probiotic

Hatching rate: Minimum hatching rate was recorded in PRO_0 (48.56±0.80) where all probiotic treatments showed better result than that of non-probiotic treatment (Figure 3). Maximum hatching rate was found in treatment PRO_2 (78.65±4.17). PRO_3 showed the second highest hatching rate (73.06±3.21) following PRO_1 (52.41±5.06).

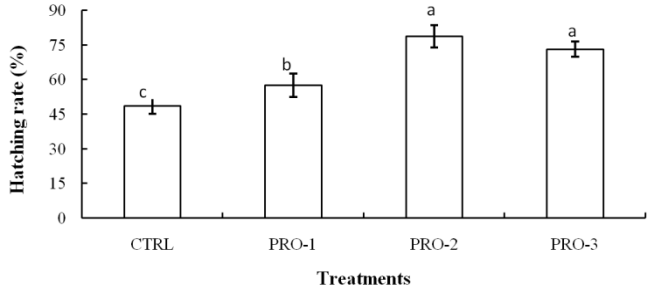

Fig.3. Comparison of hatching rate of eggs produced by female O. *pabda* reared under different levels of probiotic

Survival rate: After 60 days of experimental period the survival rate was found to be 67.43±2.78, 73.62±3.29, 78.29±2.84, 75.33±2.89 respectively inPRO_0, PRO_1, PRO_2, and PRO_3 (Table 4). There has no significant difference among all the treatments. The highest result performed by $PRO_2$and worst results performed by PRO_0.

Table 3. Effect of probiotic on the growth performance of O. *pabda*

	PRO_0	PRO_1	PRO_2	PRO_3
Initial Weight (g)	25.23 ± 0.60^a	25.37 ± 0.37^a	24.51 ± 1.0^a	23.77 ± 1.89^a
Final weight (g)	32.49 ± 0.60^c	37.69 ± 1.13^{ab}	41.27 ± 2.30^a	34.28 ± 1.93^{bc}
Initial Length (cm)	16.5 ± 0.50^a	16.84 ± 0.13^a	16.78 ± 0.18^a	16.5 ± 0.40^a
Final Length (cm)	19.55 ± 0.30^b	19.46 ± 0.08^b	20.26 ± 0.32^a	19.55 ± 0.23^b
Weight gain(g)	7.26 ± 1.20^c	12.32 ± 1.0^b	16.76 ± 2.76^a	10.95 ± 2.53^{bc}
FCR	2.34 ± 0.07^a	2.13 ± 0.14^{ab}	2.01 ± 0.18^b	1.98 ± 0.23^b
PER (%)	0.33 ± 0.06^b	0.56 ± 0.05^b	0.82 ± 0.15^a	0.46 ± 0.09^b
SGR (% /day)	0.53 ± 0.03^c	0.67 ± 0.04^{ab}	0.76 ± 0.05^a	0.62 ± 0.06^b

Mean ± SD values with different letters in each row are significantly (P<0.05) different.

Table 4. Effect of different levels of probiotic on the reproductive performances of O. *pabda*

	PRO_0	PRO_1	PRO_2	PRO_3
Fecundity	1138.55 ± 152	1408.98 ± 121	1376.4 ± 193	1275.06 ± 263
Ova (g/bw)	43.80 ± 6.49^c	45.86 ± 2.20^b	49.90 ± 4.71^a	47.91 ± 4.36^b
Ova diameter (mm)	1.32 ± 0.07^b	1.28 ± 0.03^b	1.34 ± 0.01^a	1.34 ± 0.005^a
Fry survival (%)	67.43 ± 5.41^b	73.62 ± 3.62^b	78.29 ± 3.70^a	75.33 ± 4.82^a
Death larvae (%)	32.57 ± 5.2^c	26.38 ± 3.4^b	21.71 ± 2.6^a	24.67 ± 3.1^b
Deformed larvae (%)	5.48 ± 3.2^b	3.21 ± 2.6^b	1.85 ± 1.5^a	3.95 ± 1.8^b
Fry Length gain(mm)	54.07 ± 1.27^b	56.86 ± 3.23^a	57.62 ± 3.84^a	53.55 ± 1.8^b

Mean ± SD values with different letters in each row are significantly (P<0.05) different.

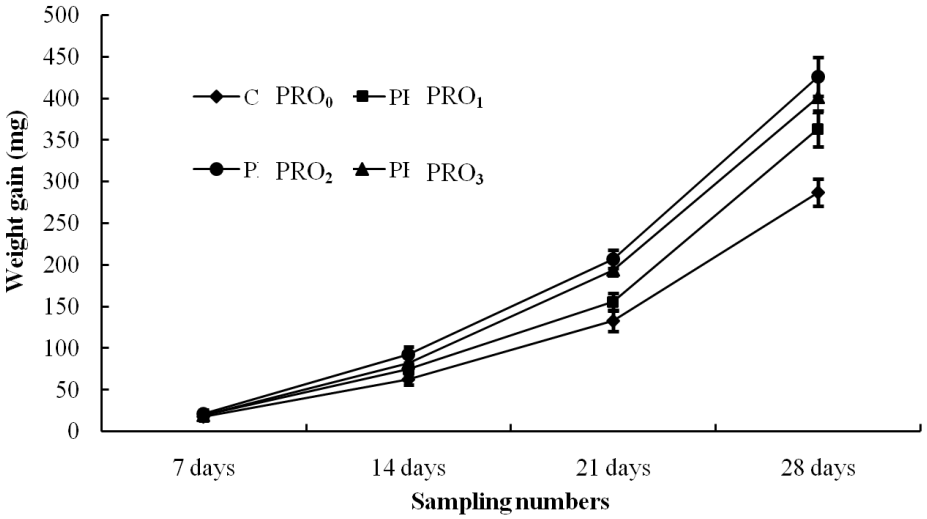

Fig. 4. Comparison of gain in weight of larvae of O. *pabda* produced from broodfish reared under different treatment level of probiotic

Growth performances of *O. pabda* larva

The weekly average length and weight of *O. pabda* larvae produced by the broodfish of four different treatmentswere measured where three different levels of probiotic were supplied. The average initial length of the fry were 11.68±0.73 mm, 12.69±0.58 mm, 12.82±0.80 mm and 13.03±0.73 mm respectively in PRO_0, PRO_1, PRO_2, and PRO_3. The final average length of the fry was 73.36±1.76 mm, 80.06±1.42 mm, 83.24±1.98 mm and 77.59±2.94 mm respectively. And the initial weight of the fry was 18.70±0.49 mg, 19.06±0.71 mg, 19.36±1.17 mg and 19.48±0.64 mg respectively in PRO_0, PRO_1, PRO_2, and PRO_3. The average final weight of the fry was 366.63±52.84 mg, 373.32±56.52 mg, 426.05±23.94 mg and 401.31±4.38 mg respectively in PRO_0, PRO_1, PRO_2, and PRO_3. The growth patterns of the larvae under different treatments are shown in (Figure 4). The fish fed with the probiotic feeds recorded significantly higher (P<0.05) survival, weight and length of fry. The length of fry also exhibited significant differences (P<0.05) within the probiotic feed-fed fish. All the probiotic-fed fish exhibited significantly lower (P<0.05) average numbers of dead fry compared with fish fed the control feed. The average numbers of deformed fry were found to be significantly higher (P<0.05) in probiotic free feed-fed fish and lower in fish fed the probiotic feed (Table 4).

Physico-chemical condition

During the experimental period temperature, dissolved oxygen and pH data were recorded which were in suitable range. Temperature (°C), pH and dissolved oxygen (mg/L) of water in tanks under different treatments were ranged between 26.5°C-29.8°C, 8.4-7.1, 4.6-5.9 (mg/L) respectively. The data were recorded in every fifteen days interval.

DISCUSSION

Assessment of reproductive performances

The results of this study demonstrated the incorporation of probiotics in feed favorably influenced the reproductive performance of *O. pabda* in terms of high fecundity, high GSI, high fry survival, higher average weight and length, reducing fry mortality and deformity of fry. The dietary administration

of an indigenous spore-forming *Bacillus* probiont resulted in an elective probiont colonization and proliferation in the host digestive tract (Rengpipat *et al.,* 2000). The findings for growth in terms of weight gain of brood fish indicate that there was a significant difference among the fishes treated with 0, 0.6, 0.8 and 1.00 g probiotic/kg feed. Similar results were also observed by (Ghosh *et al.,* 2007) who conducted an experiment on the female live bearing ornamental fish obtaining significant variation among different treatments. In this study there were no significant variations among GSI values of different treatments. However, in case of fertilization rate PRO_2 showed significant difference compared to PRO_0 whereas there was no variation among probiotic treatments. It may be due to the fact that there may have some influential effect of probiotic on fertilizing capacity of eggs and sperms. This study is demonstrated that probiotic supplemented diets also influenced the hatching rate where PRO_2 showed the highest performance compared to PRO_1 and PRO_0. It may be due to the increase of bacterial load on their digestive tract which protects them from pathogenic attack secreting some beneficial enzymes. Besides these, non-pathogenic and diverse adherent microbiota present on the eggs would probably be an effective barrier against colony formation by pathogens on fish eggs (Olafsen, 1998).

The higher survival rate of *O. pabda* also showed in PRO_2 in contrast to PRO_1 and PRO_0 may cause interaction with the intestinal probiotic bacteria which produce B group vitamins. These results corroborate with the findings of (Ghosh *et al.,* 2007) who reported that the synthesis of vitamin B_1 and B_{12} by the probiotic bacterial strain, *Bacillus subtilis* could have accounted for the reduced numbers of dead fry in four species of livebearing ornamental fish fed diets containing *B. subtilis*. In addition, it can be said that the lactic acid might have enhanced the production of inhibitory substances against the pathogenic organism. This is an agreement with the findings of (Gilberg *et al.* 1997) who reported a reduced cumulative mortality after feeding probiotic mixed feed with *V. anguillarium*. These observations are supported by the findings of (Ketola *et al.,* 1998) who reported that Thiamin (Vitamin B_1) can reduce the mortality of progeny in the Atlantic salmon. Probiotic bacteria established in the gut enhance broodstock and larval nutrition by synthesizing essential nutrients (proteins and essential fatty acids) and enzymes (amylase, protease and lipase) (Irianto & Austin, 2002). Probiotic bacteria in the fish intestine enhances host enzyme secretion by the superior maturation of fish intestinal secretory cells (Ghosh *et al.,* 2007, 2008), which increases the digestive efficacy of the complex proteins and lipids included in the diet, thus increasing the rate at which they can be assimilated by the host animal. This finding is similar to that of obtained by (De Schrijver and Ollevier, 2000), who investigated protein digestion in juvenile *Scophthalmusmaximus* and showed that supplementation of the diet with a potential probiont, *Vibrio proteolyticus*, resulted in increased digestion and absorption of protein, particularly in the distal portion of the gastrointestinal tract. Probiotic bacteria also produce B group vitamins (Goldin and Gorbach, 1992), and the production and supply of B vitamins and certain unknown stimulants (Coves *et. al.*1990) could have played a key role in the elevated reproductive performance of the probiotic feed-fed fish. Proteins and fatty acids are very important constituents of the yolk and their presence in diet consequently supports good oocyte development and maturation and a higher rate of vitellogenesis (Dahlgren, 1980). Besides the regulation of reproductive physiology, essential fatty acids also supply energy to sustain the spawning activities.

Assessment of growth performances

In the present experiment growth rate of the larvae was higher by using of probiotic which is related to the growth rate and feed utilization of farmed fish, hybrid tilapia (Genç *et al.* 2007a), Crucian carp (Xu *et al.,* 2009), African catfish (Essa *et al.,* 2011), rainbow trout (Salamatdoustnobar *et al.,* 2011). The effects of probiotic have been studied in many aquatic animals. Improvement of the growth has been reported by feeding of *Bacillus* spp. in the tilapia (Aly *et al.,* 2008), *Catla catla* (Bandyopadhyay and Mohapatra, 2009), *Labeo rohita* (Ghosh *et al.,* 2003), sea cucumber (*Apostichopusjaponicus)* (Zhang *et al.,* 2010), *Fenneropenaeusindicus* (Ziaei-Nejad *et al.,* 2006), *Macrobrachiumrosenbergii* (Keysami *et al.,* 2007) and *Penaeusmonodon* (Rahiman *et al.,* 2010). The growth rate of larvae increased significantly in PRO_2 compare to other treatments because of the digestive efficacy. Besides these the results also in harmony with that the growth of African catfish (*Clariasgariepinus*) (Al-Dohail *et al.,* 2009) and *Macrobrachiumrosenbergii* (Venkat *et al.,* 2004) fed the dietary *Lactobacillus* spp. significantly increased. Low SGR (Specific Growth Rate) was found in PRO_3(1.0g/kg) in spite of being used highest probiotic compound and less FCR (0.8g/kg). This is may be due to the fact that many bacteria release some toxic substances along with the production of more enzymes which may retard the growth or other parameters. This result is in agreement with the report of (Ghosh *et al.,* 2008), which showed that the use of higher concentration of the probiotic did not always lead to better performances of growth. However, no significant improvements of weight gain or the SGR were observed in rainbow trout (Merrifield *et al.,* 2010b) and *Epinepheluscoioides* (Sun *et al.,* 2010) fed with Bacillus spp. supplemented in diets. The reason may be the high dose of Bacillus spp. was not able to induce significant growth improvement of aquatic animals. Conversely, high levels of prebiotics may yield harmful influences on the performance and health status of fish (Olsen *et al.,* 2001). Although, (Genç *et al.,* 2007a) found positive effects of tested prebiotic on growth rate in African catfish (*C. gariepinus*), however, (Peterson *et al.,* 2010) did not observe improvement regarding weight gain in juvenile channel catfish (*Ictaluruspunctatus*) by adding Bio-MOS® prebiotic. Recently, (Hernández *et al.,* 2012) also reported that the use of commercial prebiotics (FLAVOXIN® and UNIWALL MOS 50®) at 2g / kg diet for each has a positive effect on survival of silver catfish (*Rhamdiaquelen*), without modifying growth parameters. So, it is important to define the probiotic levels administered to fish to avoid overdosing and under dosing with resultant lower efficacy and unnecessary costs.

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