



## Effects of probiotic on the growth performance of Pabda (*Ompok pabda*)

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**Abstract.** The research was conducted to assess the effects of probiotic on the growth performance of Pabda (*Ompok pabda*) at laboratory condition for a period of three months. The experiment had two treatments (T<sub>1</sub>: use of artificial diet; T<sub>2</sub>: use of artificial diet with probiotic), each with two replications. Water quality parameters including temperature (°C), pH, dissolved oxygen (mgL<sup>-1</sup>), ammonia (mgL<sup>-1</sup>), nitrite (mgL<sup>-1</sup>) and nitrate (mgL<sup>-1</sup>) were monitored regularly. They did not show any significant ( $p > 0.05$ ) differences between the treatments, and were suitable for Pabda fish throughout the culture period. Growth of fish was measured prior to stocking, every 15 days interval throughout the trial and after the trial. Initial length and weight were 8.11±0.56cm and 2.60±0.58g in T<sub>1</sub>, and 8.11±0.48cm and 2.60±0.50g in T<sub>2</sub>. Growth of fish was attributed to higher length gain (LG: 2.9±0.18 cm) and weight gain (WG: 1.38±0.09 g) in T<sub>2</sub> than T<sub>1</sub> (LG: 1.12±0.26 cm; WG: 1.05±0.15g). Low food conversion was determined in T<sub>2</sub> (1.65) than T<sub>1</sub> (2.37) after the trial. This indicates the better effects of probiotic on growth of Pabda in T<sub>2</sub>. Moreover, probiotic influenced the survival rate of Pabda with minimizing FCR. Therefore, it was revealed that probiotic might have a significant role in the growth of Pabda as an addition of supplementary diet.

**Keywords:** Probiotic, *Ompok pabda*, FCR, Survival rate

### Introduction

Bangladesh has ranked 3rd in inland open water capture fish production and 5th in aquaculture production (FAO 2022). Over the last three decades, the fish production in Bangladesh has increased more than six times (DoF 2022). Development in the aquaculture sector is one of the reasons for increasing fish production over the last few decades. Though Bangladesh possesses abundant fisheries resources but they have never been utilized properly on a scientific basis. The production of fish per hectare in this country is still lower than some of the fish producing countries of the world. This is due to lack of proper knowledge on the scientific fish culture and management practices used by the country like China and Vietnam.

Pabda (*Ompok pabda*) commonly known as 'butter catfish' is a freshwater species native to India, Bangladesh, Pakistan, and Myanmar (Mukhopadhyay and Ghosh 2007). Like other small indigenous species (SIS), this species is also distributed at all types of freshwater habitats including ponds, lakes, floodplains, oxbow lakes and semi-closed water bodies in Bangladesh (Ahmed 1981). It is well preferred for consumption as the species is delicious and has relatively few bones. The species is listed as an endangered fish species in Bangladesh.

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Fish disease is a common phenomenon in aquaculture. To address these setbacks, antibiotics have been widely used for fish disease management in the aquaculture system. In the past few years, indiscriminate use of antibiotic has led to the occurrence of antibiotic resistant (Hossain *et al.* 1999). Probiotic are in particular the live microorganisms which confer a health benefit on the host during consumptions of adequate amounts. Probiotic bacteria are now increasingly used in aquaculture to overcome antibiotic induced disease resistance, degradation of water quality and growth of farmed fish (Duncan and Klesius 1996). Although, probiotics are commonly used in shrimp and some cultured fish to prevent the disease outbreak in Bangladesh, the function is unknown in pabda culture system so far. It refers to harmless live normal flora/microorganisms that provide a health benefit on the host, when administered in adequate amounts and it also leads to have nutritional advantages. According to Fuller (1989), probiotics are live microbial feed supplements which beneficially affect the host by improving its intestinal microbial balance. Probiotics, lactic acid bacteria and *Bacillus* spp. as 'bio-friendly agents' can be introduced into the culture environment to control and compete with pathogenic bacteria as well as to promote the growth of the cultured organisms. The use of beneficial bacteria (probiotics) to displace pathogens by competitive processes is being used in the animal industry as a better remedy than administering antibiotics and is now gaining acceptance for the control of pathogens in aquaculture (Promya and Chitmanat 2011). However, nowadays, probiotics are becoming an integral part of the aquaculture practices for improving growth and disease resistance and obtaining high production (Nayak 2010, Rohani *et al.* 2022) and the concept of maintaining the health of fish through the best possible nutrition is well-accepted in modern fish farming (Kiron 2012, Jahan *et al.* 2021).

However, in the recent years, Pabda culture has been extended in Bangladesh and it is increasing in the northern part of Bangladesh as well. Nevertheless, its culture is not being well practiced due to scarcity or lack of appropriate culture technology. It is noted that there is no information on the growth of Pabda by using probiotics and artificial feed so far. Therefore, the present study was focused on the effects of providing probiotics with artificial diets on the growth performance of pabda in monoculture at laboratory condition.

### Materials and Methods

**Experimental site, experimental design and management of aquarium:** The experiment was carried out in aquaria at the laboratory of Department of Fisheries Management, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur for a period of three months from 1 October to 31 December. A completely randomized block design was followed to conduct the experiment. The experiment had two treatments assigned as T<sub>1</sub> and T<sub>2</sub> with two replications for each. In T<sub>1</sub>, artificial diet (prepared with Carbohydrate 30%, Protein 30 %, Fibre 6 %, Fat % and Moisture 12%) was provided ,whereas artificial diet and probiotic (0.2%) mixed with adequate water were applied in Treatment 2 (T<sub>2</sub>). Stocking density of Pabda fingerlings was the same (20 fingerlings in each aquarium) in both treatments. The size and shape of each aquarium used in this experiment was 25.4 × 45.7 × 25.4 cm<sup>3</sup> and rectangular, respectively having no inlet and outlet facilities. Each aquarium was filled in 20 liter tap water. The aquariums were aerated with using aerator and water was replaced every day.

**Fingerlings collection and management:** Fingerlings of Pabda were collected from a private hatchery named as Hihatchery in Dinajpur district. Fingerlings were transported with aerated

holding tanks to the laboratory at Hajee Mohammad Danesh Science and Technology University, Dinajpur. The individuals were acclimated in a holding tank for a week. After acclimation, fish were transported to aquariums for growth trial. No feed was given on first day. From the second day of stocking, feed was given to the tank at a rate of 5% of body weight of the fish. Water quality parameters (DO, pH, temperature, ammonia, nitrite and nitrate) were monitored daily using a multi-parameters probe. The tank and aquaria were cleaned every day using siphoning system. Twenty (20) fingerlings was stocked in each aquarium. Before stocking fingerlings in each aquarium, length and weight of each individual were measured.

**Feeding of fingerlings:** Fingerlings of Pabda were fed twice daily at a rate of 5% of body weight of the fish during the acclimation and trial period for three months to evaluate the growth performance of Pabda. For feeding, artificial diet (prepared with Carbohydrate 30%, Protein 30 %, Fibre 6 %, Fat % and Moisture 12%) was provided in treatment 1 ( $T_1$ ), whereas artificial diet and probiotic (0.2%) mixed with adequate water were applied in Treatment 2 ( $T_2$ ). Dietary feed ingredients were ground using a laboratory grinder and then blended into a homogenous doughy matter by adding water (Teimouri *et al.* 2013). Tongy Feed Meal Company Ltd., Bangladesh uses the above mentioned feed ingredients as an artificial diet.

**Sampling of fish:** During the experimental period, the fish samples were caught at every 15 days interval with net for measuring the growth (length and weight) of fish. Lengths of the fingerlings were measured with measuring scale and weight with weighing balance. After measuring the length and weight, fingerlings were slowly released to the aquarium.

**Determination of water quality parameters:** Water quality parameters namely, pH, temperature, dissolved oxygen, ammonia, nitrate and nitrite were measured in the morning (between 7:00-8:00 AM) and in the afternoon (between 16:00 to 17:00 PM) at every 7 days interval during the experimental period. Water temperature ( $^{\circ}\text{C}$ ), Dissolved oxygen ( $\text{mgL}^{-1}$ ), pH, ammonia ( $\text{mgL}^{-1}$ ), nitrate ( $\text{mgL}^{-1}$ ) and nitrite ( $\text{mgL}^{-1}$ ) were measured with Celsius thermometer, dissolved oxygen meter (Model DO-5509), pH meter (HANNA instruments, model HI 98107), ammonia test kit (HI 3824), nitrite test kit (HI 3873) and nitrate test kit (HI 3874), respectively.

**Estimation of growth parameters:** Growth is the progressive increase in the size (length and weight) of fish. Growth of the fish were measured at 15 days interval and at the time of final harvest. During each sampling, fish sample was taken out randomly from the water of each aquarium with the help of scope net and kept it in tissue paper containing tray for removal of water. The individual length (cm) of the fingerlings was measured with a centimeter scale fitted upon a wooden structure (precision 0.1 cm) and weight (g) was measured with an electronic balance (precision=0.1g). The total body length of the fish was measured from at the tip of the snout up to the end of the caudal fin. Length gain, weight gain, survival rate, feed conversion ratio (FCR) and specific growth rate (SGR) were calculated as follows:

Length gain (cm) = Mean final length – mean initial length

Weight gain (gm) = Mean final weight – mean initial weight

Survival rate (%) = (Number of actual fish harvested / Number of actual fish stocked)  $\times$  100

FCR = Feed applied (dry weight) / Live weight gain

SGR (% bw day<sup>-1</sup>) = [(ln final weight – ln initial weight)  $\times$  100] / Duration of the experiment (day)

**Statistical analysis:** Independent samples T-Test was performed for comparing water quality and growth parameters between the treatments. Survival and percent data were analyzed using arcsine-

transformed data, but percent values were reported. The assumptions of normal distribution and homogeneity of variances were checked before analysis. Statistical tests were carried out at a 5% level of significance using SPSS (Statistical Package for Social Science) version 16.0.

## Results and Discussion

**Water quality parameters:** In fish culture, water quality is usually defined as the suitability of water for the survival and growth of fish. Therefore, it is important to know water quality parameters and their management for better growth and survival of fish. Mean values of water quality parameters did not differ significantly ( $p>0.05$ ) between two treatments (Table 1). The mean values of water temperature were more or less close to the 24°C in both treatments. Molina *et al.* (2009) found that water temperature ranged from 19.9±0.33 to 24.82±0.12°C in tilapia ponds treated with probiotic bacteria. Mehrim (2009) observed an average temperature of 25±20°C in mono-sex Nile tilapia cultured tanks where commercial probiotics were used. Kohinoor *et al.* (2012) recorded temperature ranged from 24.60-30 °C (T<sub>1</sub>), 23.88-29.84°C (T<sub>2</sub>) and 24.50-30.30°C (T<sub>3</sub>) in stinging catfish ponds. Therefore, the mean values of temperature in this experiment were more or less identical to the findings of the above authors. The mean pH value of water was found 8.2 and 8.12 in T<sub>1</sub> and T<sub>2</sub>, respectively during study period. Mehrim (2009) found the mean pH values ranged from 7.90 to 8.19 in her mono-sex Nile tilapia research on tanks treated with different densities of Nile tilapia and commercial probiotics. Ahmed *et al.* (2022) observed the mean values of pH ranged from 8.12-8.19 in Nile tilapia fish ponds treated with some commercial probiotics and control. The recommended suitable range of pH for growth and health of most freshwater aquatic animals is 6.5-9.0 (Boyd and Tucker 1998). Therefore, the pH values of water in this research was more or less alike to the above mentioned findings and were suitable for Pabda (*O.Pabda*) fish. The mean values of dissolved oxygen (mgL<sup>-1</sup>) in this experiment were 7.0 mgL<sup>-1</sup> and 6.80 mgL<sup>-1</sup> in T<sub>1</sub> and T<sub>2</sub>, respectively. Asghar *et al.* (2022) recorded the mean values of dissolved oxygen (mgL<sup>-1</sup>) ranging from 6.28±0.14 to 6.79±0.29 in aquarium while performed an experiment on effect of probiotics on growth and health status of *Labeorohita*. Tabassum *et al.* (2021) observed the mean values of DO were ranged from 6.08 ± 0.27 to 6.79 ± 0.19 in Nile tilapia ponds treated with a common basal diet and three types of commercial probiotics. According to Boyd and Tucker (1998), fish generally feed best, grow fastest, and are healthiest when dissolved oxygen concentrations are above about 5 mgL<sup>-1</sup>. Therefore, the above findings supports the DO level (mgL<sup>-1</sup>) obtained from this experiment for growth performance of Pabda. It is noted that the dissolved oxygen levels were found in acceptable range for the aeration system and daily exchange of freshwater. The mean values of ammonia (NH<sub>4</sub>-N) concentration (mgL<sup>-1</sup>) were the same i.e. 0.5± 0.023 mg/L during the experimental period. Ahmed *et al.* (2022) observed the mean values of ammonia (NH<sub>4</sub>-N) ranged from 0.038±0.028- 0.065±0.04 mg/L in Nile tilapia fish ponds treated with some commercial probiotics and control. Bahnasawy *et al.* (2020) recorded ammonia (NH<sub>4</sub>-N) concentration (mgL<sup>-1</sup>) ranged from 0.04±0.01 to 0.11±0.02 mg/L in aquariums of Nile tilapia treated with different levels of probiotics (EM.1® and control). Therefore, the mean values of ammonia (NH<sub>4</sub>-N) concentration (mgL<sup>-1</sup>) found from this experiment are comparable to the findings of the above authors and these values were existed due to exchange of water daily. The mean values of nitrate (NO<sub>3</sub>-N) concentration (mgL<sup>-1</sup>) in two treatments were 3.0±0.094 (T<sub>1</sub>) and 2.8±0.91 (T<sub>2</sub>) in this experiment which are more or less similar to the findings of Bahnasawy *et al.* (2020) who assessed the nitrate (NO<sub>3</sub>-N) concentrations (mgL<sup>-1</sup>) as 2.62±0.41- 4.12±0.75 mgL<sup>-1</sup> in aquariums of Nile tilapia where effects of different levels of probiotics (EM.1®) with control were compared. The mean values of nitrite (NO<sub>2</sub>-N) concentration ((mgL<sup>-1</sup>) were more or less the same i.e. 0.09mg/L± 0.04 and 1 ± 0.04 (mgL<sup>-1</sup>), in

T<sub>1</sub> and T<sub>2</sub> treatments, respectively during the experimental period. This might be due to aeration and exchange of freshwater daily in aquariums. Tabassum *et al.* (2021) observed the mean values of nitrite (NO<sub>2</sub>-N) concentration ((mgL<sup>-1</sup>) ranged from 0.84±0.07 to 2±0.14 in Nile tilapia ponds compared a common basal diet with three types of commercial probiotics. Zabidi *et al.* (2021) observed the the mean values of nitrite (NO<sub>2</sub>-N) concentration ((mgL<sup>-1</sup>) ranged from 0.8 ± 0.31-2.70±0.3 mgL<sup>-1</sup> in aquariums of Red Hybrid Tilapia (*Oreochromis* spp.) fingerlings in a biofloc system treated with three different probiotics and their combination. Therefore, the values of nitrite (NO<sub>2</sub>-N) concentration ((mgL<sup>-1</sup>) of water in this research were within the range observed by the above mentioned authors and were suitable for the growth performance of Pabda (*O.Pabda*) fish.

**Table 1. Mean (SD) values of recorded water quality parameters in two treatments**

Variables	Treatments		ANOVA Significance (P value)
	T <sub>1</sub>	T <sub>2</sub>	
Temperature (°C)	23.45 ± 1.39	23.48±1.42	NS
pH	8.20 ± 0.27	8.12±0.28	NS
Dissolved oxygen (mg L <sup>-1</sup> )	7.0 ± 0.88	6.8 ± 0.96	NS
Total NH <sub>3</sub> -N (mg L <sup>-1</sup> )	0.5 ± 0.023	0.5 ± 0.023	NS
NO <sub>3</sub> -N (mgL <sup>-1</sup> )	3.0 ± 0.94	2.8 ± 0.94	NS
NO <sub>2</sub> -N (mgL <sup>-1</sup> )	.09 ± 0.04	1 ± 0.04	NS

NS, Values are not significantly ( $p > 0.05$ ) different based on Independent-Samples T-Test.

**Growth performance of Pabda (*O.Pabda*) fish:** Mean growth performance of Pabda fish under two different treatments after 3 months are presented in Table 2. Individual final length (cm), Individual final weight (g), Individual length gain (cm), individual weight gain, specific growth rate (% bw d<sup>-1</sup>), and survival rate (%) were significantly ( $p < 0.05$ ) higher and feed conversion ratio (FCR) was lower in treatment 2 (T<sub>2</sub>) where artificial diet mixed with probiotics was provided to fish as feed than treatment 1 (T<sub>1</sub>) where only artificial diet was provided to fish as feed (Table 2). From this experiment, it was evident that probiotic supplementation with artificial diet increased the growth of *O.pabda*. This might be due to the fact that artificial diet mixed with probiotic improved feed intake and feed utilization of Pabda fish that might be reflected by their better survival rate, weight gain and specific growth rate (SGR% bw d<sup>-1</sup>) with lessening the FCR. Tabassum *et al.* (2021) found significant improvements in water qualities, growth performance, feed utilization, hematological parameters, intestinal microbial load and morphology of Nile tilapia, *Oreochromis niloticus* with probiotic supplemented treatments than the control group where no probiotics were used. Asghar *et al.* (2022) reported that the probiotics in fish feed lessen the FCR, increases growth parameters and immunologic level of *Labeo rohita* in aquarium. Kumer *et al.* (2019) stated that probiotics significantly enhance the survival rate, weight gain and specific growth rate (SGR% bw d<sup>-1</sup>) of reared *Labeo rohita* juvenile with minimizing FCR in tanks. Zabidi *et al.* (2021) observed higher final weight and SGR (% day<sup>-1</sup>), and lower FCR of Red hybrid tilapia fingerlings cultured in biofloc system with addition of probiotics.

**Table 2. Growth performance (weight and length; mean± SD) of pabda in two treatments over the experimental periods**

Variables	Treatments		ANOVA significance ( <i>P</i> value)
	T <sub>1</sub>	T <sub>2</sub>	
Individual initial length (cm)	8.11±0.56	8.11±0.48	NS
Individual initial weight (g)	2.60±0.58	2.60±0.50	NS
Individual final length (cm)	9.23±0.91 <sup>b</sup>	11.01±0.68 <sup>a</sup>	*
Individual final weight (g)	3.65±0.46 <sup>b</sup>	3.98±0.37 <sup>a</sup>	*
Individual length gain (cm)	1.12±0.26 <sup>b</sup>	2.9±0.18 <sup>a</sup>	*
Individual weight gain (g)	1.05±0.15 <sup>b</sup>	1.38±0.09 <sup>a</sup>	*
Specific growth rate (% bw d <sup>-1</sup> )	1.32 ± 0.04 <sup>b</sup>	1.54 ± 0.02 <sup>a</sup>	*
Feed conversion ratio (FCR)	2.37±0.02 <sup>b</sup>	1.65±0.02 <sup>a</sup>	*
Survival rate (%)	87.89±0.34 <sup>b</sup>	94.34±0.76 <sup>a</sup>	*

NS, Values are not significantly different ( $p > 0.05$ ).

\* Values with different superscript letters in the same row indicate a significant difference ( $p < 0.05$ ) based on independent samples T-Test.

Based on the experiment it was clear that probiotics mixed with artificial diet of fish lessened the FCR, increases the growth performance in terms of individual final length (cm), Individual final weight (g), Individual length gain (cm), individual weight gain, specific growth rate (% bw d<sup>-1</sup>), and survival rate (%). Therefore, it might be concluded that probiotics are potential feed ingredients and its uses with artificial diet might play an effective role in the growth performances of Pabda (*Ompok pabda*) in aquarium. Fewer treatments with replications and short duration of the experiment were the limitations for this research. In addition, the experiment was completely laboratory based. Therefore, further research might be conducted to determine the effects of probiotic in fish diet using more treatments and replications at aquarium or at field level.

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