Effects of dietary probiotics on the growth, blood chemistry and stress response of Pabda catfish (*Ompok pabda*) juveniles

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Abstract. The current study was conducted to evaluate the effects of dietary commercial probiotics on the growth, blood chemistry and stress response of *Ompok pabda* juveniles. Eighteen homogenous sized juveniles with a mean weight of 1.31±0.04 g were randomly distributed in glass aquariums and assigned 4 treatments with 3 replicates. Fish were fed experimental diets containing commercial probiotics at a level of 0%, 0.2%, 0.4% and 0.8% for treatment groups T_1, T_2, T_3 and T_4, respectively, for 90 days. At the end of trial, 10 fish from each replicate were randomly selected for blood collection through caudal puncture with heparinized syringes. Afterwards, the stress tolerance test was conducted to determine the lethal time of 50% mortality (LT_{50}) in saline water (35 ppt), which was conducted by counting dead fish every 5 minutes after first exposure. Fish in treatment groups T_2 and T_3 showed significantly lower plasma glucose levels compared to treatment group T_1. T_4 group had an intermediate plasma glucose value. Total cholesterol (T-Cho) level was significantly higher in treatment group T_4 compared to that of T_1. All measured plasma parameters (glucose, glutamyl oxaloacetic transaminase or GOT, glutamic-pyruvate transaminase or GPT, blood urea nitrogen or BUN, triglycerides or TG, and T-Cho) showed better values in probiotics supplemented treatment groups compared to the non-supplemented treatment group. In case of weight gain, 0.2% probiotics supplemented diet showed the best performance compared to the other treatments. The LT_{50} was significantly higher in T_2 compared to T_1 and T_4. T_3 group showed intermediate LT_{50} value. Finally, 0.2% dietary probiotics improve the growth, blood chemistry and stress compensation capability of *O. pabda* juveniles.
Key Words: dietary probiotics, LT_{50}, plasma glucose, stress tolerance, total cholesterol.

Introduction. Aquaculture is one of the most promising sectors in food security, contributing with almost half to the global fish production. It has been intensifying significantly since last few decades (FAO 2018). Intensive fish farming practices at high stocking densities combined with limited prophylactic control can suppress the immune defense, and consequently result in increased susceptibility to infectious diseases (Sakai 1999). Infectious diseases are key threats to aquaculture that can result in economic decrement by causing high mortality in farmed fish (Assefa & Abunna 2018). Antibiotics, vaccination and other chemicals are currently used in aquaculture in varying degrees to control the infectious diseases. However, each of these treatment methods has its drawbacks, including the suppression of the immune system, environmental hazards and food safety problems. Moreover, long-term application of certain antibiotics can develop antibiotic resistance, which has led to the ban of sub-therapeutic use of antibiotics in Europe and more stringent regulations on the application of antibiotics in lots of other countries (Hossain et al 2020). This situation enforces aquatic animal nutritionists to develop alternative strategies for disease control and health management of aquatic animals. One of the most promising alternative strategies in this regard might be the addition of immunostimulant type functional feed additives in aquafeeds. Recently, probiotics received the highest attention in these regards.

Probiotics are live microorganisms intended to produce health benefits for the host when administered in adequate amounts (Hill et al 2014). Previous reports suggested that probiotic supplementation as functional nutrients can reduce disease outbreaks by enhancing the immune system of fish and shrimp (Kim & Austin 2006; Mohideen et al 2010; Wang & Gu 2010). It also helps to ameliorate growth parameters, health performances and stress responses in fish (Al-Dohail et al 2009; Pérez-Ramos et al 2018; Rahman et al 2018; Samarawardhane et al 2018). Hematological parameters such as plasma glucose, haematocrit, haemoglobin, red blood cell count (RBC), white blood cell count (WBC), cortisol, total cholesterol (T-Cho), glutamyl oxaloacetic transaminase (GOT), glutamic-pyruvate transaminase (GPT), triglycerides (TG) and blood urea nitrogen (BUN) are considered essential indicators of blood chemistry and health of fish and have been influenced positively by probiotics and related functional feed additives (Al-Dohail et al 2009; Hossain et al 2016a; 2017; 2018). Several blood parameters like plasma glucose, cortisol and others have also been considered as stress markers in fish. Under stressful conditions, these parameters show higher values due to the altered physiological state of fish (Hossain et al 2018). Thus, through observing these blood characteristics, the stress status and the overall health condition of the fish can be determined, which is very important for a disease-free successful aquaculture venture.

Ompok pabda, territorially known as Pabda, is a small freshwater catfish pertaining to the family Siluridae of the order Siluriformes (Siddiqua et al 2000). This species is popular with the people of East India, North East India and Bangladesh (Gupta 2018). Farmers from different regions in Bangladesh are culturing the species through improved semi-intensive systems with high stocking densities, which often incur stress and diseases in fish. As the use of antibiotics and chemicals is often discouraged, alternative strategies like supplementation with health promoting feed additives must be adapted for the successful culture of the species. Application of probiotics would play an important role in this regard. Recently, Rahman et al (2018) reported increased growth and survival rate of *O. pabda* larvae fed with probiotics supplemented diet. However, there is no adequate information available on the effects of probiotics as a functional feed additive to regulate hematological parameters and stress response of this important freshwater species. Therefore, the current research was conducted to evaluate the principal hematological parameters and stress responses of *O. pabda* fed with dietary probiotics as well as to determine an optimum supplementation level to produce an effective diet, which would provide a favorable physiological condition to culture this species commercially in Bangladesh.

Material and Method

Test fish and experimental design. The experiment was conducted from October 15, 2017 to January 12, 2018 in the Laboratory of Aquaculture, Faculty of Fisheries, Sylhet Agricultural University, Bangladesh, for a period of 90 days. Pabda fry were collected from a private hatchery in Mymensingh, Bangladesh, and transported in oxygenated polythene bags from the hatchery to the university campus. Afterwards, fish were acclimated in a 500 L freshwater tank for one week before the commencement of the experiment. There was continuous aeration and the fish were fed a commercial catfish diet containing 43% protein three times per day (15% of their body weight) during this period. After being acclimatized to the new environment, homogenous size juveniles were selected. 216 fish, having a mean initial body weight of 1.31 g, were randomly allocated to twelve prepared glass aquaria following a Completely Randomized Design (CRD), 18 fish per aquarium. Four treatments with three replicates were used for the experiment. The feeding trial was carried out in 150 L glass aquaria filled with 100 L of water. Water was stored in a reservoir tank used as a source of freshwater for the aquaria. Each aquarium had continuous aeration and filtration. The treatments were labeled as T_1 (control), T_2, T_3 and T_4, with experimental diets containing 0%, 0.2%, 0.4% and 0.8% probiotics, respectively. The aquaria had a natural light regime. Weight of fish was recorded as initial weight before stocking. Water quality parameters such as temperature, dissolved oxygen, pH and salinity were determined at the beginning of the experiment and measured twice per week during the feeding trial. The behavior of Pabda catfish was also observed, especially after feeding, to monitor movement, infections, colorations and diseases.

Preparation of the experimental diets. Table 1 summarizes the chemical composition of the commercial catfish diet (ACI Company Ltd., Bangladesh) used as the basal diet in this study. The basal diet was grounded with an electric blender (Myako, India) to obtain powder. Afterward, four experimental diets were prepared by supplementing incremental levels of commercial dietary probiotics (Navio plus, Biovac, Thailand). Initially, the required amount of probiotics were thoroughly mixed with the grounded basal diet. After proper mixing, water was added gradually (35–40%) to the dry ingredients. The mixture was then passed through a kitchen type pellet machine with an appropriate diameter (0.5 mm) to prepare pellets, which were then sun-dried at ambient temperature (35^0C–40^0C) for 6 hours. Afterwards, they were packed in airtight polythene bags and stored at 4^0C until use.

Table 1

Proximate composition of the basal diet

Proximate composition	*Percentage (%)*
Moisture	11
Crude protein	43
Crude fat	8
Carbohydrates	20
Fibers	3
Ash	12
Calcium	2
Phosphorous	1

Feeding protocol. Fish were fed the experimental diets at 10% of their body weight in the 1st month of their rearing period, and the percentage was gradually reduced to 8% and 5% of their body weight in the 2nd and 3rd month, respectively. Daily ration size was divided into three equal ratios (morning at 7:00 AM, midday at 1:00 PM and evening at 7:00 PM). Uneaten feed was removed through siphoning twice a day and 50% water renewal was done at a two day interval.

Sampling and weight gain. Sampling was conducted every 2 weeks to determine the growth of Pabda catfish and to adjust the feeding rate. Growth was measured with a digital balance (CAMRY digital electrical balance, Model EK 3052, Bangladesh). After the end of the 90 days feeding trial, weight gain and percentage of weight gain were calculated by following the equations:

Weight gain = Mean final weight – Mean initial weight

$$\%\text{ of weight gain} = \frac{\text{Weight gain}}{\text{Initial weight}} \times 100$$

Blood samples collection and biochemical analysis. At the end of the feeding trial, fish were starved for 24 hours prior to the final sampling. All the fish were anesthetized with eugenol (4-allylmethoxyphenol, Wako Pure Chemical Ind., Osaka, Japan), 50 mg L^{-1}. Ten fish from each replicate were used for blood collection using 1 mL heparinized syringes (Jimi syringes and medical devices Ltd, Bangladesh). Blood was collected from the caudal vein of ten fish from each replicate tank and pooled for plasma. Plasma samples were obtained by centrifugation at 3000 x g for 10 minutes using a high-speed micro centrifuge (Capsule HF-120, Tomy Kogyo Co., Ltd., Fukushima, Japan) and kept at -20°C until analysis. Afterwards, plasma chemical parameters such as glucose, GOT, GPT, BUN, TG and T-Cho were measured spectrophotometrically using commercial kits with a semi-automated analyzer (3000 EVOLUTION, Biochemical Systems International Srl, Arezzo, Italy).

Saline water stress tolerance test. At the end of the feeding trial, the stress tolerance test was conducted to determine the lethal time of 50% mortality (LT_{50}) in saline water containing commercial table salt (NaCl). Six fish from each rearing aquarium (total 18 fish per treatment) were randomly selected and transferred into a 150 L rectangular glass aquarium with 30 L of prepared saline water (35 ppt). The time duration for 50% mortality of Pabda catfish juveniles was calculated according to Hossain et al (2016a) as follows: time to death (minutes) of a juvenile was converted to $\log_{10}$ values. When the juveniles were transferred to the saline water, they were still alive, so the survival rate was assumed to be 100% (log value was $\log_{10}100=2$). The calculation was carried out every 5 minutes after exposure. The values of survival rate were then plotted against every minute to determine the time needed for 50% mortality of the juveniles to occur in each treatment. The equation is as follows:

$Y=aX+b$, where $Y=\log_{10}$ (survival) and X is the time for individual juvenile death (minutes).

LT_{50} (X) was obtained when Y=1.7, since $\log_{10}50=1.7$.

Statistical analyses. Statistical analyses were performed using one-way analysis of variance (ANOVA). Duncan's multiple range test was used to determine differences between treatment means at a significance rate of $P<0.05$. The standard deviation of treatment means was also estimated. All statistical analyses were carried out using the Statistical Package for Social Science (SPSS) version 23.

Results and Discussion

Water quality parameters. The values of water quality parameters were considered to be within optimal values for freshwater catfish culture (Cline 2019) (Table 2).

Table 2

Water quality parameters for 90 days experiment

Water quality parameters	*Values ± SD*	*Reference values*
Temperature (^{O}C)	19.33±2.52	18.33-26.67
Dissolved oxygen (mg L^{-1})	5.37±0.21	3-10
pH	6.97±0.23	6-8
Salinity (ppt)	0	0

Weight gain. The weight gain is presented in Figure 1. In comparison to the control, the percentage of weight gain was significantly higher in T_2, followed by T_3 and T_4. However, there were no significant difference observed between T_3 and T_4 ($P>0.05$). A lower percentage of weight gain was found in T_1 (Control) (Figure 1).

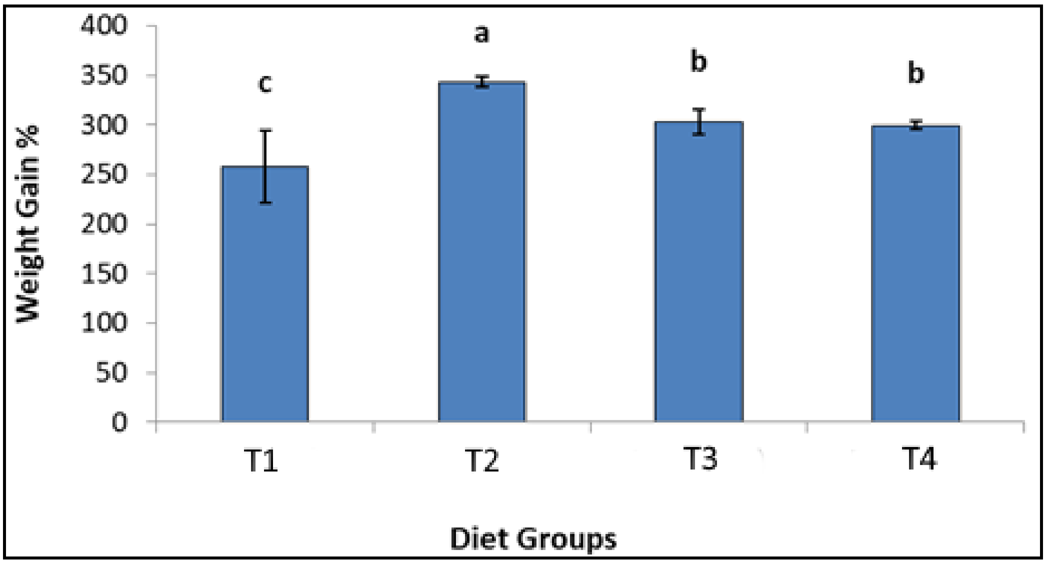

Figure 1. Weight gain of juvenile Pabda catfish (*Ompok pabda*) fed test diets for 90 days. T1 - basal diet without probiotics; T2 - basal diet with 0.2% probiotics; T3 - basal diet with 0.4% probiotics; T4 - basal diet with 0.8% probiotics. Different letters above columns suggest significant differences (P<0.05).

Hematological parameters. Dietary treatments had no significant effect (P>0.05) on plasma chemical parameters of fish, except for plasma glucose and T-Cho. At each supplementation level, fish fed probiotics supplemented diets showed a relatively lower plasma glucose level compared to those fed the control diet. The lowest glucose level was in T_2 (49.7±1.5 mg dL^{-1}), followed by a gradual increase in T_3 (51.3±6.1 mg dL^{-1}) and T_4 (66.7±6.6 mg dL^{-1}) (Table 3).

In comparison to the control diet group, significantly higher T-Cho was found in the treatment group T_4 (240.3±8.4 mg dL^{-1}) where fish were fed with the highest level of dietary probiotics (8 g kg^{-1}). T_2 and T_3 showed intermediate T-Cho levels (T_2: 216.0±4.2 mg dL^{-1} and T_3: 232.7±12.5 mg dL^{-1}) (Table 3).

Table 3

Blood parameters of juvenile Pabda catfish (*Ompok pabda*) fed test diets for 90 days

Parameters	*Diet groups*			
	T_1	*T_2*	*T_3*	*T_4*
Glucose (mg dL^{-1})	$77.7±4.9^b$	$49.7±1.5^a$	$51.3±6.1^a$	$66.7±6.6^{ab}$
T-Cho (mg dL^{-1})	$189±10.1^a$	$216±4.2^{ab}$	$232.7±12.5^{ab}$	$240.3±8.4^b$
BUN (mg dL^{-1})	<5	<5	<5	<5
GOT (IU L^{-1})	36±19.1	23.7±7.5	24±1	27±4.2
GPT(IU L^{-1})	13.3±4.3	<10	<10	11.7±2.2
TG (mg dL^{-1})	300.7±58.7	>500	>500	418±49.5

Note: different superscripts in the same row show significant differences (P<0.05). T-Cho - total cholesterol; BUN - blood urea nitrogen; GOT - glutamyl oxaloacetic transaminase; GPT - glutamic-pyruvate transaminase; TG - triglyceride. T_1 - basal diet without probiotics; T_2 - basal diet with 0.2% probiotics; T_3 - basal diet with 0.4% probiotics; T_4 - basal diet with 0.8% probiotics.

Numerically, higher GOT (36.0±19.1 IU L^{-1}) and GPT values (13.3±4.3 IU L^{-1}) were observed in the control group. However, no significant differences were observed for GOT and GPT values among the treatment groups. Fish fed with different levels of probiotics supplemented diet showed increased TG levels (>500 in T_2; >500 in T_3; and 418.0±49.5 mg dL^{-1} in T_4) compared to those of the control group (300.7±58.7 mg dL^{-1}). In addition, the BUN level did not significantly differ among the diet groups (Table 3).

Salinity stress tolerance test. The values of LT_{50} against saline water shock were obtained by regression analysis and are presented in Figure 2. Supplementation of probiotics significantly improved the saline water stress tolerance of Pabda catfish, and significantly higher LT_{50} value (40.5 min) was obtained for fish in T_2, followed by T_3 (37.5 min). Significantly lower LT_{50} value (30.5 min) was observed in T_1, which was not significantly different from that of T_4.

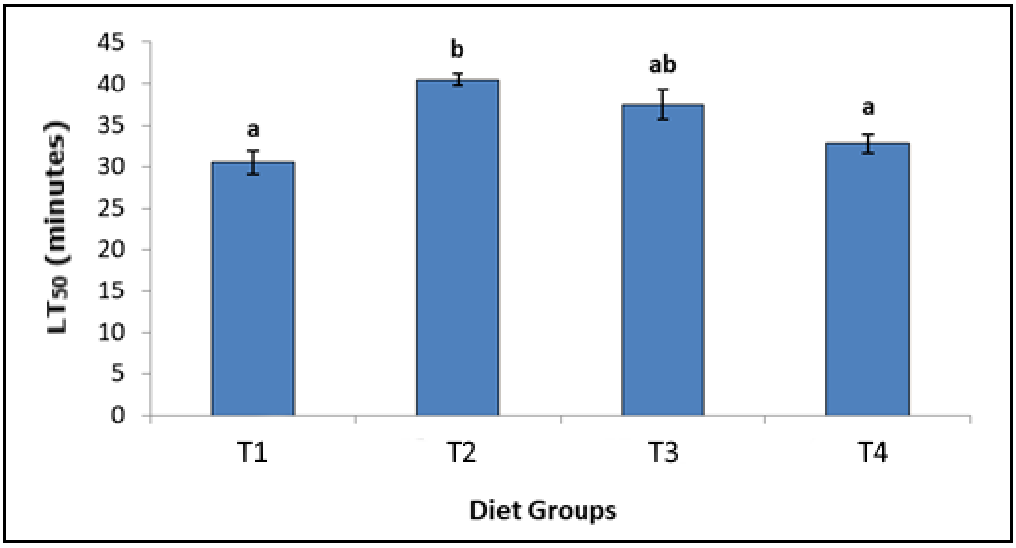

Figure 2. LT_{50} (minutes) for Pabda catfish (*Ompok pabda*) exposed to saline water. Different letters above columns show significant differences (P<0.05). T1 - basal diet without probiotics; T2 - basal diet with 0.2% probiotics; T3 - basal diet with 0.4% probiotics; T4 - basal diet with 0.8% probiotics.

Probiotics have increased demand in eco-friendly aquaculture to accelerate fish production in a healthy environment (Wang et al 2008; Nayak 2010). Currently, many probiotic products extracted from different species of bacteria including *Bacillus* spp., *Lactobacillus* spp., *Carnobacterium* spp., *Enterococcus* spp. and yeast (*Saccharomyces cerevisiae*) have gained popularity in aquaculture as alternatives to antibiotics because of environment friendly characteristics (Talukder Shefat 2018). It is already proved that probiotics have significant effect on growth, digestibility, survival, hematological parameters, immune resistance and stress responses of many commercially important fish species (Nayak et al 2007; Al-Dohail et al 2009; Dawood et al 2015; Rahman et al 2018; Ali et al 2018; Mehrabi et al 2018; Samarawardhane et al 2018). However, little information was found regarding probiotics administration in Pabda catfish diet.

In the present study, the weight gain of *O. pabda* was significantly higher when dietary probiotics were used. Similar increased growth performances due to probiotics supplementation were also reported for other fish species, like *Clarias gariepinus* (Al-Dohail et al 2009; Ayoola et al 2013), *Oreochromis niloticus* (Aly et al 2008), *Catla catla* (Bandyopadhyay & Das Mohapatra 2009) and *Labeo rohita* (Ghosh et al 2003). The main reasons behind this increased growth and feed utilization performances in the current study might be due to the increased digestive enzyme activity, elevated health status and stimulation of gastric development and/or enzymatic secretion in probiotics supplemented groups. Dose is an important consideration while supplementing functional nutrients in aquafeeds (Hossain et al 2020). The present study revealed that more than 0.2% probiotics in the diet are responsible for a lower growth of *O. pabda* juveniles. A higher concentration might not be able to maintain the overall body physiology of fish and it could create disturbances in carbohydrate and fat metabolism. Probably *O. pabda* could not adapt to more than 0.2% probiotics, and as a result, weight gain was reduced. Ghosh et al (2008) also showed that the use of a higher concentration of probiotics does not always lead to better growth performances of ornamental fish.

In the present study, water quality parameters such as temperature, pH, DO and salinity were found in optimum levels for the culture of Silurid catfish (Southworth et al 2006; Munni et al 2013; Cline 2019). No negative effects on water quality parameters in the current study were observed due to probiotics supplementation in aquafeed, which also resembled with the findings of Wang & Zirong (2006), who reported that no important effects on water quality parameters were observed when common carp (*Cyprinus carpio*) was fed with probiotic photosynthetic bacteria and *Bacillus* sp.

Blood parameters are being used as an efficient and sensitive index for the physiological and pathological status as well as well-being of fish (Hossain et al 2017). Hematological parameters obtained in the present experiment were within the normal range compared with those of previous findings (Hossain et al 2016a,d; 2017). In the present study, there were no significant differences in plasma chemical parameters of *O. pabda* in different treatments, except for those of plasma glucose and T-Cho. Probiotics supplementation reduced plasma glucose content and a significantly lower value obtained in T_2. Similar reduced plasma glucose was observed in *C. gariepinus* fed with a probiotic supplemented diet (Al-Dohail et al 2009). The decreased level of plasma glucose was also observed in red sea bream (*Pagrus major*) juveniles fed with dietary functional supplements like inosine and inosine monophosphate (Hossain et al 2016d). Plasma glucose is commonly considered to be one of the stress indicators in fish, high glucose levels often indicating a higher stress status of fish (Eslamloo et al 2012). The body physiology and metabolic activities increase in fish when they remain under stress conditions. Therefore, fish may require a higher level of plasma glucose under stressful conditions to recover energy. In the present study, the lower level of plasma glucose in probiotics supplemented diet groups indicated that dietary probiotics ensured an optimum and healthy physiological condition in fish, reducing the environmental stress. Interestingly, the level of plasma glucose within treatments increased gradually with the increasing percentage of probiotics. Therefore, it could also be said that high levels of probiotics might not be suitable for the optimal physiological condition of *O. pabda* catfish.

In the present study, probiotics supplemented diet groups showed significantly higher T-Cho values compared to the control group. Cholesterol is an important component of cell membranes (Cheng & Hardy 2004). Probiotics are made of bacterial cells, where the bacterial cytoplasmic membrane is composed of phospholipid bilayer. Therefore, the gradual incremental level of T-Cho observed in fish fed probiotics supplemental groups might be due to the gradual incremental level of dietary probiotics in supplemented groups. Higher T-Cho levels were also reported in red sea bream (*P. major*) fed diets with a functional supplement, guanosine monophosphate (Hossain & Koshio 2017). In contrast, decreased T-Cho was also observed in some fish species fed diets with probiotics and other functional supplements (Al-Dohail et al 2009; Hossain et al 2017; 2018). The supplementation of probiotics reduced plasma GOT (or aspartate aminotransferase, AST) and GPT (or alanine aminotransferase, ALT) levels, which are often used for the evaluation of liver function. These are released in the blood during injuries or damage to the liver cells (Lemaire et al 1991). Similar reduced GOT and GPT levels were observed in fish fed functional supplements (Hossain et al 2016a; Hossain & Koshio 2017). Lower values of these parameters in probiotics supplemented groups in the current study indicated that probiotics induced an optimal physiological function and better liver health condition of fish compared with the probiotic-free control group.

Stress is one of the emerging issues in aquaculture activities, which may affect hormonal secretion rates, intermediary metabolism, immunity and nutrient utilization (Li et al 2009). Diets supplemented with different nutritional or immunostimulant functional supplements such as probiotics, GMP, nucleotides and others have significant effects on stress tolerance capacity and immune response of fish (Nayak et al 2007; Beck et al 2015; Dawood et al 2015; Hossain et al 2016a,c; 2017; 2018). Unfortunately, there were no similar studies found on the effects of dietary probiotics on stress responses of juvenile *O. pabda*. Therefore, the current study reveals valuable information on the effects of probiotics on stress tolerance in Pabda catfish. All three probiotics treated groups (T_2, T_3 and T_4) showed a higher value for LT_{50} compared to that of the control group. Moreover, T_2 offered the highest LT_{50} time, indicating an increased stress tolerance capacity of Pabda catfish fed with probiotics (0.2%) supplemented diet. This result confirmed the findings of Hossain et al (2016a,b), where guanosine monophosphate and adenosine monophosphate supplemented diets increased the stress resistance in red sea bream (*P. major*) after exposure to low salinity waters. Higher LT_{50} in *O. pabda* fed probiotics supplemented diet indicates the healthy status of Pabda catfish.

Conclusions. The present study demonstrated that the dietary supplementation of probiotics positively influenced the growth, blood characteristics and stress tolerence of Pabda catfish. Considering the overall performance, it can be concluded that 0.2% dietary probiotics might be the optimum supplementation level for improved physiological and hematological status, a congenial stress compensation capability of *O. pabda* juveniles. However, further experiments should be designed to determine the efficacy of probiotics as functional supplements on immune responses, including controlled disease challenges to elucidate the integrated disease defense mechanisms that may be affected by probiotics in Pabda catfish.

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