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Effects of probiotic supplementation on the growth performance of Thai silver barb (*Barbonymus gonionotus*) (Bleeker, 1850) fry

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ABSTRACT

A six-week long feeding trial was performed with fifteen rectangular glass aquaria (35 L capacity) containing 180 fish (average weight 1.02±0.3 g) to evaluate the effects of probiotic supplementation on the growth performance of Thai silver barb, *Barbonymus gonionotus* fry. The fry were fed twice-a-day at 8-10% of their body weight. Three available commercial probiotics were added with commercial crumbled feed in three forms; (i) feed + 3% 'Biofav aqua' (T1), (ii) feed + 3% 'NavioPlus' (T2), (iii) feed + 3% 'Eskalina' (T3), whereas, water additive probiotic 'ARIAKE3' (T4) was added directly to water and feed without probiotics was control (T5). Results revealed that water quality parameters were best in T4 compared to others. Net weight gain (3.46 g), percent weight gain (361.67%), (SGR) (3.63%) and survival (100%) were significantly higher ($p<0.01$) in T4. FCR (2.06) ($p<0.05$) and PER (1.34) ($p<0.01$) were significantly lowest but FCE (0.48) was significantly ($p<0.05$) highest in T2 compared to others. Control showed lower growth and feed utilization efficiency compared to probiotics supplemented treatments. Whole body protein, lipid and carbohydrate content were also found highest in T4. In the present study, the addition of probiotics in the regular diets significantly increased the growth and survivability of this commercially important fish. However, considering the growth, food efficiency and the water quality index, the water additive probiotic showed more beneficial effects for the monoculture of the selected fish which may be used in commercial farming for better growth performance of *B. gonionotus*.

Keywords: Thai silver barb, growth performance, probiotics, *Bacillus*, water quality

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1 Introduction

Over the years, aquaculture has been expanded, diversified and intensified with advanced technologies and thus, these protein-contributing sector has been contributing significantly to the world's gross animal-derived protein (FAO, 2016). However, the regional and world-wide demand of fish nutrition is still below the requirement for the growth population. Developing countries such as Bangladesh, India, Vietnam and similar countries with potential aquaculture scopes are facing common shortcomings like high price of the commercial feeds and quality feed ingredients, undesirable growth performance due to lack of available quality feeds and deterioration of water quality. These have led the aquaculturists to search for alternative feed formulation with probiotics to improve the feed efficiency through higher digestibility and nutrients utilization. Besides, development of drug-resistant pathogens due to indiscriminate use of antibiotic is another growing concern (Gao et al., 2012; Cabello, 2006). The aquaculture products having residual effects of antibiotics can cause human health problem (Cabello, 2006) and may also lead loss of beneficial gastrointestinal microbiota (Jernberg et al., 2010). Therefore, an alternative approach using probiotics in aquaculture is gaining more acceptance in recent years.

Probiotics, which are bacteria in the feed, have received much more attention in the field of fish feed technology due to its multifunctional properties (Dawood and Koshio, 2016). Probiotics exclude pathogen by producing extracellular inhibitory compounds, creating iron-limiting niche for the other pathogenic microorganisms (Travers et al., 2011; Verschuere et al., 2000; Qi et al., 2009). It also produces several enzymes that can break down the indigestible compounds potentially present in the supplementary feed; thus, it leads the higher feed efficiency in the aquaculture (EL-Haroun et al., 2006; Cruz et al., 2012). Several previous reports have suggested that probiotic supplement in the feed may prevent disease outbreaks by enhancing the immune system of fish and shrimp (Kim and Austin, 2006; Wang and Gu, 2010), may reduce rearing costs by improving the feed efficiency (Mohapatra et al., 2011; Peterson et al., 2011) and can reduce water pollution by lessening residual feed waste in water column (Peterson et al., 2011; Zhou et al., 2009).

Thai silver barb or Java barb, *Barbonymus gonionotus* (Bleeker, 1850), a fresh water species found in the South-east Asia including Bangladesh, India, Thailand and Vietnam. It has become popular due to its high productivity and delicious taste. Moreover, since silver barb reaches marketable size within four months, it is an ideal fish for culture in the large numbers of seasonal ponds of Bangladesh (Gupta, 1990). Silver barb responds well to comparatively low cost and simple management practices (Akhteruzzaman, 1991) and thus making it suitable for poor and marginal farmers. Recently, the intensive culture of *B. gonionotus* is gaining importance in the fish-growing countries with artificial feeds (Mohanta et al., 2006). However, farmers are still facing challenge with the high price of the commercial pelleted feeds with limited efficiency for fish growth and maintaining good water quality (Nandi et al., 2012). Therefore, we conducted an in-situ growth-based experiment for the first time in Bangladesh and its region to determine the efficacy of various probiotics in the water and feed supplement for better growth and feed utilization of *B. gonionotus* fry.

2 Materials and Methods

2.1 Aquaria management

A set of fifteen glass aquaria (capacity 35 L) filled with 30 L clean ground water were used for fish rearing for six weeks. The aquaria were served with artificial aeration and kept under room temperature (24–30 °C). Healthy silver barb (*B. gonionotus*) fry (average body weight 1.02±0.3 g) were collected from the Fisheries Field Research Complex of Bangladesh Agricultural University, Mymensingh, Bangladesh and transported to the experimental facilities. The fry were reared for one week in aquaria with oxygen flow to acclimatize to the laboratory conditions and fed crumble floating feed (Quality Feeds Ltd., Dhaka, Bangladesh) at the rate of 8 – 10% of fish body weight twice daily. Percentage (%) wise ingredient's composition of the basal diet is shown in Table 1.

Table 1. Ingredient composition of the basal diet (as per specification of the manufacturer)

Name of ingredients	Composition (%)
Fish meal	50
Soybean meal	10
Full fat soybean	10
Mustard oil cake	10
Wheat bran	15
Rice bran	4
Vitamin and mineral premix	1

2.2 Preparation of probiotics-supplemented feed

Four commercially available probiotics i.e., P1 (probiotic 1): BIOFAV AQUA (feed additive, Novartis, Bangladesh), P2 (probiotic 2): NAVIOPLUS (feed additive, ACI animal health, Thailand), P3 (probiotic 3): ESKALINA (feed additive, Eskayef, Dhaka, Bangladesh) and P4 (probiotic 4): ARIAKE 3 (water additive, Indonesia) were selected based on the composition and purchased from local market. P1 consisted of wide range of Gram positive *Bacillus* spp. along with other bacteria (e.g., *Paracoccus* sp.) and fungi (e.g., *Aspergillus* sp.), P2 hosted comparatively fewer species of *Bacillus* sp. mixed with *Saccharomyces cerevisiae*, the P3 was composed of organic *Spirulina*, a blue green algae. The water additive, P4 also contained mixed *Bacillus* sp. in small quantity with calcium carbonate and starch. Rice starch (water from the boiling rice) was taken, cooled to room temperature and 1% of each feed additive probiotic powder was mixed well separately with it to make the probiotic suspension. Then, previously mentioned commercial pellet feed was mixed well with the probiotic suspension randomly and dried inside the room using a fan. The feed was kept in airtight plastic bags, stored in the refrigerator at 10 °C and used for feeding of the fingerlings (Jahan et al., 2016). The formulated diet was named as T1 for P1, T2 for P2 and T3 for P3 while the ready-to-use diet was T4 for P4. The basal diet (without probiotics) served as Control. The proximate composition of formulated feeds and body tissue was determined according to AOAC (2000).

2.3 Feeding experiment for silver barb

Replicates were maintained with the aquarium for each of the treatment (four formulated feeds and one control diet). *B. gonionotus* fry (12 individuals) were incubated into aquarium contained 30 L of ground water. At the starting of the incubation (1 h of acclimatization), three individuals were harvested and sacrificed for the fish body proximate composition analysis. The rearing was started in the last week of September (moderate rainy season). The fish were fed with the formulated and control diet. The feeding rate was as follows: twice daily at the rate of 10% of fish body weight for three weeks followed by 8% for the next three weeks. All aquaria were cleaned daily by 75% water exchange by siphoning during removal of fish feces and uneaten feed.

2.4 Fish growth and feed utilization

Once a week, ten fish were randomly sampled using a fine meshed scoop net, anesthetized with commercially available anesthetic agent MS-222 (Tricaine methanesulfonate) and the average body weight (wet basis) was measured using an electric balance (Model-HT224R, Shinko Denshi Co. Ltd, Tokyo, Japan). Growth and feeding efficiency were determined by evaluating several growth and nutrient utilization indices, including percentage weight gain, specific growth rate (SGR), feed conversion ratio (FCR), feed conversion efficiency (FCE) and protein efficiency ratio (PER). The growth parameters and feed utilization were calculated as follows:

$$WG_{wet} = F_W - I_W \tag{1}$$

where, WG_{wet} = wet weight gain (g), F_W and I_W are final and initial weight (g)

$$SGR = \frac{F_W - I_W}{T} \times 100 \tag{2}$$

where, SGR = specific growth rate (% d^{-1}), F_W = final weight (g), and I_W = initial weight (g), and T = days of feeding

$$FCR = \frac{TDF}{LWG} \tag{3}$$

$$FCE = \frac{LWG}{TDF} \tag{4}$$

$$PER = \frac{LWG}{CPI} \tag{5}$$

where, FCR, FCE, and PER denote feed conversion ratio, feed conversion efficiency, and protein efficiency ratio. TDF = total dry feed consumption (g), LWG = live weight gain (g), and CPI = crude protein intake (g).

2.5 Water quality determination

Water quality parameters *viz.*, dissolved oxygen (DO), temperature, free ammonia (NH_3) and pH were monitored weekly throughout the incubation period after twelve hours of water exchange. The DO values were recorded using a portable DO meter (Lutron DO-5509, Taiwan) and denoted as milligram per liter (mg L^{-1}). Water temperature was measured using a thermometer and denoted as °C. Water pH of the individual aquarium was recorded using a portable pH meter (EcoSense, Model-10A, USA). Free ammonia was measured using Nessler method (Shugar et al., 2001) using Spectrophotometer (HACH, model-DR 2800, USA).

2.6 Data processing and analysis

The effect of various feed treatments on the fish growth and proximate composition was tested by one-way ANOVA using SPSS 20 (IBM, USA) at the 95% level of confidence. The post-hoc analysis was also performed to compare between one-to-one of the treatments using Duncan Multiple Range Test (Duncan, 1955).

3 Results

3.1 Proximate composition of feeds

Commercial fish feed (crumble) supplemented with P1 contained 37.48% crude protein, 6.80% crude lipid, 26.89% carbohydrate, 11.45% ash, 13.18% moisture and 4.20% crude fibre. Fish feed mixed with P2 showed 36.03% crude protein, 7.20% crude lipid, 22.75% carbohydrate, 13.99% ash, 15.23% moisture and 4.80% crude fibre. Again, basal feed mixed with P3 contained 36.72% crude protein, 3.60% crude lipid, 29.30% carbohydrate, 10.97% ash, 15.61% moisture and 3.80% crude fibre. Basal diet (without probiotic) used for the treatments (T4 and control) contained 35.62% crude protein, 6.50% crude lipid, 20.17% carbohydrate, 12.18% ash, 21.07% moisture and 4.46%

Table 2. Average proximate composition of the diets (% moisture basis)

	CP (%)	CL (%)	Carbohydrate (%)	Ash (%)	Moisture (%)	CF (%)
T1	37.48±1.72	6.80±0.50	26.89±1.25	11.45±1.46	13.18±0.46	4.20±0.67
T2	36.03±1.30	7.20±0.78	22.75±1.33	13.99±1.90	15.23±0.68	4.80±0.56
T3	36.72±1.61	3.60±0.89	29.30±1.79	10.97±1.38	15.61±0.52	3.80±0.39
T4	35.62±0.8	6.50±1.4	20.17±1.3	12.18±1.0	21.07±1.2	4.46±0.4
Control	35.62±0.8	6.50±1.4	20.17±1.3	12.18±1.0	21.07±1.2	4.46±0.4

Values presented are mean±SE (n = 3). CP, CL and CF denote crude protein, crude lipid and crude fibre, respectively

Table 3. Growth responses of *B. gonionotus* fry in different treatments for 6 weeks

	NWG (g)	WG (%)	SGR (% d^{-1})	FCR	FCE	PER	Survival (%)
T1 †	2.54b±0.12	274.67b±8.17	3.14b±0.05	2.39a±0.09	0.42b±0.01	1.12b±0.04	94.45c±2.77
T2	2.73b±0.01	302.00b±8.54	3.32b±0.05	2.06b±0.05	0.48a±0.01	1.34a±0.03	100.00a±0.00
T3	2.82b±0.23	282.33b±5.45	3.19b±0.03	2.38a±0.03	0.42b±0.01	1.15b±0.01	97.22b±2.77
T4	3.46a±0.01	361.67a±26.67	3.63a±0.13	2.34a±0.06	0.43b±0.01	1.19b±0.03	100.00a±0.00
Control	2.56b±0.08	263.67b±10.47	3.07b±0.06	2.36a±0.04	0.42b±0.01	1.19b±0.02	86.11d±2.78
LSD	0.228	25.609	0.137	0.11	0.015	0.058	3.914
Sig. level	*	*	*	*	*	*	*

† Values presented are mean±SE (n = 3). In a column, figures with same letter or without letter do not differ significantly whereas figures with dissimilar letter differ significantly (as per DMRT at 5% level of probability)

Table 4. Proximate composition (% wet wt. basis) of *B. gonionotus* at the beginning and end of the experiment

	Moisture (%)	CL (%)	CP (%)	Ash (%)	CF (%)	Carbohydrate (%)
Initial †	78.46 ± 0.31	2.40 ± 0.12	13.59 ± 0.24	4.80 ± 0.13	0.40 ± 0.02	0.35 ± 0.01
T1	72.59±0.29b	3.11±0.07b	14.25±0.05b	2.18±0.03b	2.19±0.04	5.69±0.43b
T2	73.64±0.21a	3.17±0.12b	14.31±0.15b	2.21±0.02b	2.09±0.06	4.59±0.18c
T3	73.99±0.02a	3.05±0.05b	14.51±0.03ab	2.42±0.06a	2.04±0.05	3.99±0.06d
T4	70.96±0.14c	3.32±0.12a	14.75±0.03a	2.20±0.02b	1.98±0.01	6.78±0.28a
Control	73.99±0.13a	2.26±0.08ab	13.69±0.23c	2.21±0.05b	2.22±0.13	5.64±0.48b

† Data present the mean ± SE (n = 3). In a column, figures with same letter or without letter do not differ significantly whereas figures with dissimilar letter differ significantly (as per DMRT at 5% level of probability). CL, CP and CF denote crude lipid, crude protein and crude fibre, respectively

crude fibre. The proximate composition of diets are shown and compared with control in Table 2.

3.2 Growth and feed utilization of fish

The feed supplemented with probiotics significantly increased the growth of fish ($p<0.05$). The control (basal diet) increased fish growth by 263.67±10.47% at the end of the rearing period (six weeks) while the other four probiotic supplemented diets furthered this figure greatly (Table 3). For instance, the percent weight gains of *B. gonionotus* fry were 274.67±108.17%, 302.00 ±108.54%, 282.33±105.45%, 361.67±1026.67% in T1, T2, T3, T4, respectively. In terms of specific growth rate, similar trend was observed as 3.14±100.05 g, 3.32±100.05 g, 3.19±100.03 g, 3.63±100.13 g and 3.07±100.06 g in T1, T2, T3, T4 and Control, respectively. Among the probiotic diets, P4 (T4) performed relatively higher for the growth of silver barb ($p<0.05$) followed by P2 (T2), P3 (T3), and P1 (T1) (Table 3).

The feed conversion efficiency (FCE), however, was not correlated with the growth profile (body weight) ($r = 0.06$). For instance, the highest FCE was obtained in the supplement of T2 (0.48±0.01) followed by T4 (0.43±0.01), T1 (0.42±0.01), Control (0.42±0.009), and T3 (0.42±0.006), respectively. Similarly, the protein utilized by the fish fry was also higher for T2 as indicated by the higher protein efficiency ratio (PER) (1.34±0.03) while others were significantly lower (1.12±0.04, 1.15±0.01, 1.19±0.03 and 1.19±0.02 for T1, T3, T4 and Control, respectively)

($p<0.05$) (Table 3). The study results also showed that FCR was significantly lowest ($p<0.05$) in fish fed on the probiotics treated diet in T2 compared to the other groups. The effects of T4 and T2 also prominent in the survival rate of *B. gonionotus* fry during the incubation period, which resulted zero mortality. On other hand, other treatments with formulated diets T1 and T3 supported survival rate of 94.45±2.77% and 97.22±2.77%, respectively. Without any probiotic feeds, the survivability was (86.11±2.78%), which was significantly lower than the formulated diets ($p<0.05$).

3.3 Effects on fish body compositions

The two important components, such as carcass protein and carbohydrate contents in fish body were 13.59±0.24% and 0.35±0.01%, respectively before the feed was supplemented. The body composition showed that protein and carbohydrate deposition increased in every treatment at the end of the rearing period (13.69 – 14.75% protein and 3.99 – 6.78% carbohydrate) (Table 4). Following similar pattern, other feed- induced nutritional composition, such as lipid and crude fiber also increased significantly at the end of the six-week incubation period (Table 4). Expectedly, moisture and body ash decreased proportionally with the increase of the rest of proximate composition (Table 4). Among the feed treatments, the highest protein and lipid contents were found in T4 (14.75 ±0.03 and 3.32 ±0.12, respectively) compared to others (14.51±0.03 ∼ 13.69 ±0.23 and 3.17±0.12 ∼ 2.2±0.08, respectively) ($p<0.05$). Similarly, T4 contributed a higher amount of carbohydrate (6.78 ±0.28%) to the fish nutritional composition than for the other feed applications (Table 4). The range of crude fiber content was 1.98 to 2.22% where no significant difference ($p>0.05$) was found among the treatments.

3.4 Water quality parameters

Water temperature varied slightly (24±2.20 °C to 30.70±2.08 °C) during the study period. The maximum temperature was recorded as 30.70±2.08 °C at Day 0 in T1, while the minimum was 24.00±2.20 °C at the end of the rearing in the same treatment (Table 5). The values of dissolved oxygen varied from 4.9±1.78 to 7.4±1.40 mg L^{-1}. The highest dissolved oxygen value was 7.4±1.40 mg L^{-1} at the end of the experiment in T4 and the lowest value was 4.8±1.62 mg L^{-1} at the beginning of the incubation period in Control (Table 5). The water pH ranged from 7.6±0.44 to 8.4±0.73 during the study period. The highest pH value was 8.4±0.73 at the end of the experiment in the Control while the lowest pH value was 7.6±0.44 at the end of the total cultivation period in T2 (Table 5). The concentration of free ammonia varied from 0.01±0.01 to 0.16±0.05 mg L^{-1}. The highest free ammonia was 0.16±0.05 mg L^{-1} at the end of the study in control and the significantly lower value was 0.01±0.01 mg L^{-1} ($p<0.05$) at the initial stage of the incubation period in T4 (Table 5).

4 Discussion

The nutritional composition and quantity in the feeds influences the utilization of those feeds for the growth of fish. Insufficient energy in diets causes the waste of protein due to the increased proportion of dietary protein used to convert energy and also subsequently the produced ammonia from the protein can deteriorate the water quality (Mohanta et al., 2007). In the current study, all the diet treatments were not significantly differ with each other (e.g., protein 35.62 – 37.48%) and all that were in suitable level for carp and cichlid species (Mridha et al., 2017). Among the emerging technologies, use of probiotics in the supplementary diets has been potentially useful in terms of feed efficiency (Dawood and Koshio, 2016).

In the present study, higher growth performance (e.g., weight gain and the specific growth rate) was observed in silver barb fry fed with the probiotics-supplemented diets (T1, T2, T3 and T4) than the control. Several probiotics have been demonstrated growth promoters in the recent aquaculture practices for the carp fish (Nayak et al., 2007; Kumar et al., 2006) and other similar freshwater fishes (Ridha and Azad, 2011). The probiotic microorganisms enhance digestion processes of aquatic animals through production of extracellular enzymes, such as proteases, lipases; also, they have intended abilities for supplying necessary growth factors, such as fatty acids, vitamins and other congenital compounds (Vine et al., 2006). The improvement in growth may be, however, related to the species-specific performances of the probiotics. For instance, in a two month of period with the rearing of *Labeo rohita*, a feed supplemented with *Bacillus subtilis* increased growth by 9.8 g compared to the basal diet (Aly et al., 2008). However, this gain in growth furthered by 10.84 g while *Lactobacillus acidophilus* was used as sole probiotic and slowed down to 7.51 g in the use of co-probiotics of *B. subtilis* and *L. acidophilus* (Aly et al., 2008). In this current study, no single *L. acidophilus* amended diet was used. However, among the treatments, T4 containing three *Bacillus* sp. e.g., *B. amyloliquefaciens*, *B. pumilus* and *B. licheniformis* showed significantly ($p<0.05$) higher growth and survival rate than that of other diets containing mixed bacterial agents. Experimentally, *B. amyloliquefaciens* strain, isolated from the activated sludge, was also a very efficient nitrite-N cleaner, which was able to completely remove 10 mg L^{-1} of nitrite-N present in the simulated aquaculture water within 24 h (Xie et al., 2013). *B. pumilus* isolate was found strongly inhibitory against the marine bacterial pathogens *Vibrio alginolyticus*, *V. mimicus* and *V. harveyi*, and weakly inhibitory against

Table 5. Water quality parameters of different treatments observed at weekly intervals during study period

	Day 0†	Day 7	Day 14	Day 21†	Day 28	Day 35	Day 42†
Temperature (°C)							
T1	30.70±2.08 ns	29.34±2.30	28.70±1.83	27.10±1.73 ns	26.30±2.03	25.10±1.54	24.00±2.20 ns
T2	30.26±1.70	29.26±2.25	28.86±2.07	27.36±2.07	26.46±1.90	25.12±1.86	24.26±1.91
T3	30.54±2.03	29.40±1.73	28.90±1.80	27.40±2.43	26.52±2.08	25.22±2.03	24.30±2.12
T4	30.46±1.60	29.56±1.86	28.26±2.03	27.56±1.80	26.16±1.75	25.37±1.90	24.13±1.89
Control	30.30±2.03	29.23±2.42	28.30±1.63	27.25±2.03	26.27±2.2	25.09±2.07	24.50±1.76
Dissolved O_2 (mg L^{-1})							
T1	6.8±1.80 ns	6.0±1.03	6.4±2.14	6.6±1.70 ns	6.2±1.88	5.8±1.41	6.4±1.25 ns
T2	6.4±1.42	6.3±1.47	5.8±1.20	6.3±1.55	5.9±1.45	6.3±1.43	6.6±1.40
T3	5.5±1.71	5.9±2.03	6.2±2.25	6.1±1.31	6.7±2.30	6.5±1.35	6.7±1.35
T4	7.0±2.10	6.5±2.07	7.2±1.25	6.8±2.1	7.3±1.45	6.9±1.12	7.4±1.40
Control	4.8±1.62	5.0±1.03	4.9±1.78	5.5±1.75	6.3±2.1	6.1±2.01	6.4±2.23
pH							
T1	8.2±0.50 ns	8.1±0.40	7.8±0.65	8.0±0.50 ns	8.1±0.33	8.0±0.38	8.1±0.65 ns
T2	7.7±0.35	8.2±0.54	8.3±0.72	7.9±0.44	8.2±0.81	7.9±0.44	8.1±0.70
T3	8.1±0.22	7.8±0.36	7.7±0.80	8.0±0.71	7.9±0.72	8.0±0.61	8.2±0.81
T4	8.2±0.25	7.9±0.67	8.4±0.73	8.3±0.34	8.2±0.60	8.1±0.80	7.8±0.75
Control	7.8±0.12	8.3±0.80	8.2±0.10	7.9±0.56	8.3±0.57	8.4±0.91	8.1±0.80
Free ammonia (mg L^{-1})							
T1	0.08±0.12b	0.03±0.02	0.04±0.03	0.05±0.04 ab	0.04±0.01	0.06±0.04	0.05±0.001 b
T2	0.04±0.02a	0.09±0.01	0.06±0.01	0.04±0.03 ab	0.05±0.02	0.02±0.01	0.04±0.001 b
T3	0.03±0.12a	0.01±0.03	0.03±0.02	0.06±0.02 bc	0.03±0.01	0.04±0.02	0.05±0.002 b
T4	0.03±0.02a	0.01±0.01	0.02±0.03	0.03±0.02 a	0.02±0.03	0.01±0.01	0.01±0.002a
Control	0.02±0.01a	0.10±0.02	0.08±0.04	0.07±0.05 c	0.13±0.06	0.14±0.03	0.16±0.005 b

† Indicates the selective days taken for statistical analysis with post-hoc test (Duncan at 95% level of confidence). Different letters indicate statistically significant among the treatments on the same day. ns = Not significant

V. parahaemolyticus in cross-streaking assays on solid medium (Hill et al., 2009) and *B. licheniformis* is recognized as a potential probiotic for its ability to produce subtilin-like antibiotics which is very active against food-borne pathogens (Halami, 2019). The effect of mono and mixed bacterial agents was supported by similar report (Aly et al., 2008). T4 also contained additional supplements into the probiotic mix, which were calcium carbonate ($CaCO_3$) and starch. The starch can be used as dietary carbohydrate source for fish nutrition (Sørensen et al., 2010) while trace amount of $CaCO_3$ in the probiotics could serve as the inorganic supplement for the water quality (Wills et al., 2016). Overall, the combination of the water adding probiotic, ARIAKE3 supplemented in T4 is thought to perform better in the case of *B. gonionotus* growth than dietary probiotics.

In addition, *Bacillus* sp. became one of the dominant probiotic agents in the diet supplements as reported for variety of fish rearing (Decamp et al., 2008; Kumar et al., 2006) since this bacterial agent could improve digestibility and immunity of the aquaculture species (Daniels et al., 2010). However, yeast additional to *Bacillus* sp. might also increase food digestibility in fish gut (Abdel-Tawwab et al., 2008; Abu-Elala et al., 2013). In this study, T2 contained one yeast (*Saccharomyces cerevisiae*) in addition to *Bacillus* sp. and the higher FCR, FCE and survival obtained in the supplement of T2 indicated that yeast might have significant role in the digestibility and immunity of Thai silver barb. The results are in agreement with the findings on Nile tilapia (Ayyat et al., 2014), salmonids (Merrifield et al., 2010) and common carp (Yanbo and Zirong, 2006). The positive effect of T4 and T2 in the fish body weight gain, food conversion efficiency and survivability was also further supported by the proximate composition of the fish body (Table 3).

In the present study, the control experiment was maintained with standard range of water temperature (30 °C – 24 °C), which was not changed with the other diet treatments (Table 5). This temperature was usually optimum for Thai barb during the autumn to late autumn season in sub-tropical country like Bangladesh (Moniruzzaman et al., 2015). Similarly, pH also remained undulated but in the range of neutral to alkaline over the 42 d of rearing period.

The acidic pH in aquaculture pond arise concern due to the rapid microbial decomposition of the excessive food debris and metabolites of the feed residue (Bosma and Verdegem, 2011). The present studies did not show such acidic environment in the aquaria system. However, the lower amount of free ammonia in T4 (0.01±0.02 mg L^{-1} at day 42, median: 0.02 mg L^{-1} over the full rearing period) indicated that the diets (0.04 – 0.16 mg L^{-1}) other than T4 might lead pollution in the rearing water. Usually the concentration of unionized ammonia should not exceed more than 0.025 mg L^{-1} in aquaculture water (Alabaster and Lloyd, 2013). The higher digestibility in T4 and T2 as indicated by the higher FCR, FCE and PER (Table 2) might explain the low production of free ammonium in the water. In the case of T4, it was even more relevant with the lowest amount of toxic ammonia while the proximate fish composition (e.g., protein and lipid, Table 4) yielded in higher rate in the supplement of T4 probiotics. In the *Bacillus* sp. dominating probiotic-supplemented fish culture, the water quality might be improved through converting organic substances (e.g. organic matter, fish and feed debris) into the non-toxic simpler forms (Verschuere et al., 2000). However, higher load of organic matter in fish pond further imposes a higher consumption of dissolved oxygen resulting shortage of DO for the growing fish (Boyd, 1973). A comparatively higher level of DO (7.4±1.40 mg L^{-1} at day 42, median: 7.05 mg L^{-1} over full rearing period) in the T4 treatment indicated that the *Bacillus* sp. might facilitate the prevention of an excess depletion of dissolved oxygen. The insight mechanism of this process with the *Bacillus*-dominated diet, however, might need further investigation.

5 Conclusions

In the present study, the addition of probiotics in the regular diets significantly increased the growth and survival of this commercially important small fish. However, considering the growth, food efficiency and the water quality index, the water additive probiotic showed more beneficial effects for the monoculture of the selected fish. This first-time lab-based experiment on Thai silver barb would contribute significant insights and lead further research areas; in particular, (i) the customization of pure *Bacillus* sp. based probiotics with the other bacterial consortia (e.g., *Lactobacillus* and *Saccharomyces* sp.), (ii) the influence of selected probiotics on the immune responses of *B. gonionotus* and similar species against frequently occurring pathogenic disease, (iii) the optimization of the use of probiotic supplements with the culture techniques (mono-, poly-species) and the variable associated cost.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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