

EFFECTS OF COMMERCIAL PROBIOTIC ON GROWTH AND FEED UTILIZATION PARAMETERS OF ASIAN STINGING CATFISH (*Heteropneustes fossilis*) FINGERLINGS

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Abstract

Feeding experiment was conducted for 62-days with commercial probiotic “Navio plus” to identify and quantify its effects on growth and feed utilization parameters of Asian stinging catfish (*Heteropneustes fossilis*). Ten (10) *H. fossilis* fingerlings (3.63±0.02 g) were stocked in each aquarium and 12 aquaria were divided into four groups (3 aquaria/group). Fish groups were fed without probiotic, 2, 5 and 10g / kg probiotic which was marked as T1 (control), T2, T3 and T4 (treatments), respectively. At the end of the experiment, mean final body weight (FBW) of T1, T2, T3 and T4 was 12.92±0.01, 14.05±0.01, 15.67±0.01 and 16.21±0.01 g, respectively. Similarly, mean weight gain (WG) was 255.83±0.42, 287.05±0.27, 331.68±0.27 and 346.56±0.27%, respectively. Among these results, FBW and WG in all treatments were significantly ($P < 0.05$) higher than control (T₁), where T₄ had the highest value among the treatments. Like as FBW and WG, specific growth rate was also significantly increased in all treatment groups. In case of feed utilization parameters, feed conversion ratio and feed efficiency ratio among these diet groups remain unchanged ($P > 0.05$). However, protein efficiency ratio in T₁ (1.30±0.100) was the lowest, whereas, T₄ achieved the highest value as found in FBW and WG. Therefore, tested probiotic may be used at a dose higher than its company recommended dose to get higher growth as well as more production for profitable *H. fossilis* aquaculture in Bangladesh.

Key Words: Commercial probiotic; Aquaculture; Growth performance; Stinging catfish; Water quality.

Introduction

During the last 5 years (2013-2017), average GDP increment in fishery sector was 6.28%, whereas land-based agriculture achieved only 2.45% (DOF, 2018). However, intensification of aquaculture involves huge amount of poor-quality feed supply, water reuse and high stocking density leading to degradation of aquatic environment and fish growth retardation resulting lower production in aquaculture farms.

Asian stinging catfish (*Heteropneustes fossilis*) locally known as “shing” is widely distributed throughout the Indian sub-continent. It possesses good taste and palatability, less fat and spine, achieve marketable size within 100-120 days and act as a tonic for immune compromised patients. Lately, after falling the demand and price of tilapia (*Oreochromis niloticus*) and pangas (*Pangasius hypophthalmus*), culture of this fish has become very popular among the fish farmers. However, commercial aquaculture in Bangladesh is mainly manifested with two major constraints, one is different types of diseases like epizootic ulcerative syndrome (EUS), motile *Aeromonas* septicemia, vibriosis etc. (Aftabuddin *et al.*, 2016) and another is slow growth due to low digestibility of commercial feed (Faruk *et al.*, 2004). Likewise, *H. fossilis* farmers are facing problems due to high priced commercial pelleted feeds with lower feed utilization efficiency and their profit margins are decreasing day by day. Feed cost accounts about 60-75% of total aquaculture cost (Nahar *et al.*, 2015) and by

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current culture practices, fish farmers are trying to increase fish growth by improving feed utilization parameters (FUP) through different feed additives including probiotics (Hasan et al., 2019).

A probiotic organism can be regarded as a live, dead or component of a microbial cell, which can be administered via feed or into rearing water, benefiting the host by improving growth performance, feed utilization, immune health status, infectious disease resistance and stress responses (Nguafack et al., 2020). Probiotics have the ability to improve digestive process by enhancing beneficial bacterial population and microbial enzymatic activities, ultimately improving digestibility and absorption of nutrients towards elevating specific growth rates (SGR) and feed utilization parameter (FUP) (Suzer et al., 2008).

Different types of commercial probiotics like Organic Green™ in *O. niloticus* (Aly et al., 2008) and Calsporin® in common carp (*Cyprinus carpio*) (Mehrabi et al., 2018) improved growth and FUP in cultured species. “Navio plus” is a commercial probiotic of “ACI Company Limited, Bangladesh” which is composed of *Bacillus* (*Bacillus subtilis*, *B. licheniformis*, and *B. megaterium*), *Lactobacillus* (*Lactobacillus acidophilus* and *L. plantarum*) and *Saccharomyces cerevisiae*. In aquaculture species recommended dose of this commercial probiotic is 3-5 g kg⁻¹ of feed (company labeling) which was not yet evaluated in shing culture.

Therefore, the aim of this research was to identify and quantify the effects of Navio plus on growth and FUP modulation in *H. fossilis* fingerlings. Moreover, whether lower or higher than company recommended dose of this probiotic in *H. fossilis* culture is acceptable or not, was also evaluated.

Materials and Methods

Collection of experimental fish, probiotics and feed

Before starting, 200 *H. fossilis* fingerlings were collected from Freshwater Station, Bangladesh Fisheries Research Institute, Mymensingh. The fish were acclimatized in the experimental condition for two weeks with control diet.

A commercial probiotic “Navio plus” was collected from ACI pharmaceutical company limited, Bangladesh. According to the company label, total plate count of this probiotic was 1.0×10¹⁰ CFU g⁻¹ and specific compositions were *B. subtilis* (1×10¹⁰ CFU g⁻¹), *B. licheniformis* (1×10¹⁰ CFU g⁻¹), *B. megaterium* (1×10¹⁰ CFU g⁻¹), *L. acidophilus* (1×10⁵ CFU g⁻¹), *L. plantarum* (1×10¹⁰ CFU g⁻¹) and *S. cerevisiae* (1×10⁵ CFU g⁻¹).

Preparation of probiotic supplemented feed

To prepare the probiotic supplemented feed, 300 ml supernatant from the boiling rice was cooled at room temperature and mixed well with 2, 5 or 10 g of the probiotic powder to make the probiotic suspensions of the three different concentrations. These suspensions were mixed with 1 kg of commercial feed (Mega feed, Spectra hexa feeds limited) separately. After that, suspension absorbed commercial feeds were air dried at room temperature using an electric fan and were kept in separate plastic pots with proper labelling for daily feeding. The basal diet without probiotic was served as control and was marked as T₁, 2 g probiotic/kg as T₂, 5 g/kg as T₃ and 10 g/kg as T₄. Each diet group had three replications in separate aquaria during the feeding experiment in the wet laboratory of the Department of Aquaculture, Bangladesh Agricultural University (BAU), Mymensingh.

Fish stocking and feeding trial

After 2 weeks of acclimatization, the fish were screened for similar size (3.61± 0.02 g), each 10 apparently healthy fingerlings were distributed into 12 separate glass aquaria (63.5 × 30.5 × 38.1 cm³) of 50 L water holding capacity and provided with continuous aeration. Each 3 (three) aquaria were randomly selected for the replication of control (T₁) and treatment (T₂, T₃ and T₄) diets. The fish were fed with the above-mentioned experimental diets at 10:00 am and 5:00 pm for 60 days at 5% of their body weight.

Monitoring of water quality parameters

Water quality parameters like dissolved oxygen (DO) (mg/L), water temperature (°C), and pH were monitored throughout the study period with DO meter (LUTRON PDO-519, TAIWAN), Celsius thermometer and pH meter (EZODO, pH 5011), respectively. Total ammonia (NH₃) was determined (mg/l) by a commercial kit (ab83360, Abcam, Thailand).

Fish sampling

Fish were sampled once a week by a scoop net and body weight was measured carefully using an electronic balance. These weights were used to measure weekly weight gain (WG) and adjust the feeding rate for the following week.

Growth and FUP calculation

The following feed utilization parameters were worked out after Hasan *et al.* (2018a, b).

- ❖ IBW (g): Initial body weight = Initial weight of total fish in tank/Fish number
- ❖ FBW (g): Final body weight = Final weight of total fish in tank/Fish number
- ❖ WG: Weight gain = [(Final weight – Initial weight)/Initial weight].
- ❖ %WG: Percent weight gain = [(Final weight – Initial weight)/Initial weight] × 100.
- ❖ SGR: Specific growth rate (%/day) = [(ln final weight – ln initial weight)/days] × 100.
- ❖ FCR: Feed conversion ratio = Dry feed intake/Wet body weight gain
- ❖ PER: Protein efficiency ratio = Wet weight gain/Protein fed.
- ❖ FCE: Feed conversion efficiency = Live weight gain/Dry feed consumed

Proximate composition analysis of feed samples

The commercial feed Mega feed, before addition of the probiotic was analyzed for protein, lipid, carbohydrate, ash, moisture and crude fiber content in the Fish Nutrition Laboratory, BAU, Mymensingh. The values were calculated according to the following formulae of AOAC (1995).

- ❖ Moisture (%) = [Raw sample weight – Oven dried sample weight] / Raw sample weight] × 100
- ❖ Crude protein = 6.25 × % Total nitrogen

[% Total nitrogen = [(Milliequivalent of nitrogen (0.014) × Titrant value (ml) × Strength of HCl) / Sample weight (g)] × 100

- ❖ Crude lipid content (%) = Extracted lipid / Sample weight × 100
- ❖ Crude ash content (%) = Weight of ash / Weight of sample × 100
- ❖ Crude fiber (%) = [Weight of sample after air drying – Weight of sample after ashing / Sample weight] × 100
- ❖ Nitrogen free extracts (%) = 100 – (Moisture % + Crude protein % + Crude lipid % + Crude fiber % + Ash %)

Statistical analysis

All data were analyzed by IBM SPSS software (SPSS Inc., version 17.0, Chicago, IL, USA). One-way ANOVA followed by Duncan's multiple range test were used to compare significance of differences among the feeding groups. A *P*-value of less than 0.05 (*P* < 0.05) was considered statistically significant. Data are presented as the mean ± standard deviation (SD).

Results and Discussion

Physicochemical parameters of the aquatic environment

The results of the water quality parameters as temperature, DO, pH and NH₃ was ranged from 27–31°C, 6.5–8.4 mg l⁻¹, 6.4–8.4 and 0–0.01 mg l⁻¹, respectively (Table 1). Water quality parameters are one of the most important factors for successful aquaculture and as previously described, the beneficial effects of probiotic in water quality improvement has been proven in aquaculture. It was presumed that beneficial heterotrophic bacteria accelerate the decomposition of organic matters by establishing the management of an equilibrium of carbon:nitrogen ratio (Avnimelech and Ritvo, 2003). Queiroz and Boyd (1998) applied Biostart, a commercial *Bacillus* sp inoculum into channel catfish (*Ictalurus punctatus*) that increased different water quality parameters which ultimately influenced the health condition, survival and growth of fish compared to controls. During this experiment temperature was varied from 27 to 31°C. Water temperatures in *H. fossilis* and tilapia ponds were found to be varied from 27.90-27.49°C (Kohinoor et al., 2012) and 24- 32.8°C (Haque, 2014), which were similar to the present study condition. Growth and feeding rate is decreased when DO levels are below 1-5 mg l⁻¹, optimum being more than 5 mg l⁻¹ (Jhingran, 1988). In the present study, DO concentration in water varied from 4.9 to 7.4 mg/l which is documented to be suitable for commercial fish culture (Alam et al., 2015). The value of pH indicates acidity-alkalinity condition and called the productivity index of water body. Acceptable range of pH for fish culture is 6.5 to 8.5 whereas slightly alkaline pH is most suitable for fish farming. By contrast, acidic pH of water reduces the growth and metabolic rate and other physiological activities of fishes (Sayeed et al., 2015). In the present study, the range of pH varied from 6.4 to 8.20 which was within the most suitable range as also found by Ahmed et al. (2012) and Kohinoor et al. (2012). Unionized NH₃ is toxic to fish, while the ammonium ion (NH₄⁺) is non-toxic. In the present study, free NH₃ concentration varied from 0.10 to 0.20 mg l⁻¹. Cha et al. (2013) performed a study to configure the possible role of *Bacillus subtilis* in improving the water quality of olive flounder (*Paralichthys olivaceus*) culture environment and found that addition of probiotic bacteria into the water resulted in elimination of NH₃.

Table 1. Range of water quality parameters during the study period of 62 days

Parameters	Values range
Temperature (°C)	27 - 31
Dissolved oxygen (mg l ⁻¹)	6.5 - 8.4
pH	6.4 - 8.4
Ammonia (mg l ⁻¹)	0-0.01

Proximate composition of feed

Proximate compositions of basal diet “Mega feed” after laboratory analysis demonstrated protein, lipid, ash, moisture, fiber, carbohydrate as 36.40, 6.20, 14.06, 11.77, 4.32 and 27.25%, respectively (Table 2). However, on the bag the written ingredients like protein, lipid, ash, moisture, fiber, carbohydrate was 36, 5, 14.06, 11.77, 4.32 and 27.25%, respectively.

Table 2. Proximate compositions of “Mega feed” found after laboratory analysis in comparison to that written on the bag

Name of the major composition	Composition (%)	
	Written on bag	After lab analysis
Moisture	12	11.77
Lipid	5	6.20
Protein	36	36.40
Ash	16	14.06
Fiber	5	4.32
Carbohydrate	31	27.25

Growth performance of fish

At the end of the experiment, mean WG of fish were 9.29 ± 0.015 , 10.42 ± 0.012 , 12.04 ± 0.014 and 12.58 ± 0.011 g in T₁, T₂, T₃ and T₄ diet groups, respectively. Percent WG values were 255.83 ± 4.21 , 287.05 ± 2.75 , 331.68 ± 2.58 and 346.56 ± 3.75 in the respective tanks. Moreover, SGR values were 2.12 ± 0.002 , 2.26 ± 0.001 , 2.44 ± 0.003 and 2.49 ± 0.001 in T₁, T₂, T₃ and T₄, respectively. Table 3 clearly demonstrated WG, %WG, and SGR values in the treatment groups were significantly ($P < 0.05$) higher compared to control (T₁) and T₄ group had the highest values compared to the others.

In this study, different concentration levels of a commercial probiotic Navio plus were supplemented (T₂, T₃ and T₄) with an invariable diet in *H. fossilis* fingerlings and better growth performance (WG, %WG and SGR) were found compared to un-supplemented control group (T₁). Among different treatments, T₄ (10 g/kg probiotic administered fishes) showed the highest WG, %WG and SGR which indicated better production and daily growth compared to others. Similar type of outcomes like muscle weight, WG and SGR were observed after supplementation of commercial probiotics (AlCare®, *B. licheniformis*) in sea water Nile tilapia (Han *et al.*, 2015). In case of catfish graded levels of probiotics increased growth performances of pangas (Mahmud *et al.*, 2016) and magur (*Clarias batrachus*) fingerlings (Jahan *et al.*, 2016). The probiotic microorganisms enhance digestion processes of aquatic animals through secretion of extracellular digestive enzymes, such as protease, lipase, amylase and/or have intended abilities for supplying necessary growth factors as fatty acids, vitamins and minerals (Jang *et al.*, 2019). Better SGR is an indication of improvement in the health status and daily growth performance of shing despite the differences in the rearing methods. Similar to this result, certain duration of feeding with commercial probiotics like Proviotic® (*L. bulgaricus*) in rainbow trout (Kurdomanov *et al.* 2019) positively modulated SGR. Improvement of growth parameters is directly related to the improvement in the intestinal microflora balance, abundance and diversity after probiotic supplementation (Jang *et al.*, 2019).

Table 3. Growth performances and survival of *Heteropneustes fossilis* fed with probiotic supplemented treatment (T₂, T₃, and T₄) and control (T₁) diets, for 62 days¹

Feeding groups	IBW ²	FBW ³	WG ⁴	% WG ⁵	SGR ⁶ (% /day)	SR ⁷
T ₁	3.61±0.02	12.92±0.015 ^d	9.29±0.015 ^d	255.83±4.21 ^d	2.12±0.002 ^d	100±0.00
T ₂	3.63±0.01	14.05±0.011 ^c	10.42±0.012 ^c	287.05±2.75 ^c	2.26±0.001 ^c	100±0.00
T ₃	3.62±0.02	15.67±0.014 ^b	12.04±0.014 ^b	331.68±2.58 ^b	2.44±0.003 ^b	100±0.00
T ₄	3.60±0.01	16.21±0.010 ^a	12.58±0.011 ^a	346.56±3.75 ^a	2.49±0.001 ^a	100±0.00

¹Values with different superscript letters within the same column are significantly different ($P < 0.05$). The lack of superscript letter indicates no significant differences ($P > 0.05$).

Feed utilization parameters of fish

The mean FCR of *H. fossilis* were 1.86 ± 0.100 , 1.80 ± 0.120 , 1.63 ± 0.200 and 1.60 ± 0.130 in T₁, T₂, T₃ and T₄, respectively (Fig. 1a). Similarly, FCE was also recorded as 0.54 ± 0.030 , 0.57 ± 0.050 , 0.61 ± 0.030 and 0.62 ± 0.045 in these respective tanks (Fig. 1b). In these serially numbered tanks, PER was observed to be 1.30 ± 0.100 , 1.35 ± 0.20 , 1.49 ± 0.100 and 1.51 ± 0.020 , respectively. Significantly highest PER was found in T₄ and lowest was in T₁ (Fig. 1c). There was no significant ($P > 0.05$) variation of FCR and FCE, except PER ($P < 0.05$) among the four diet groups. In this study FCR and FCE showed slightly better result but modulation was not up to the significant levels compared to control. Similarly, probiotics were unable to modulate the FUP in different commercial species like Nile tilapia (He *et al.*, 2013) and Atlantic salmon (*Salmo salar*) (Jaramillo-Torres *et al.*, 2019). In the present study, higher protein utilization determined as PER, increased in fish maintained with probiotic supplemented diets. This result was in agreement with the findings of better PER in Nile tilapia supplemented with commercial probiotics *Streptococcus faecium* and *L. acidophilus* (Lara-Flores *et al.*, 2003).

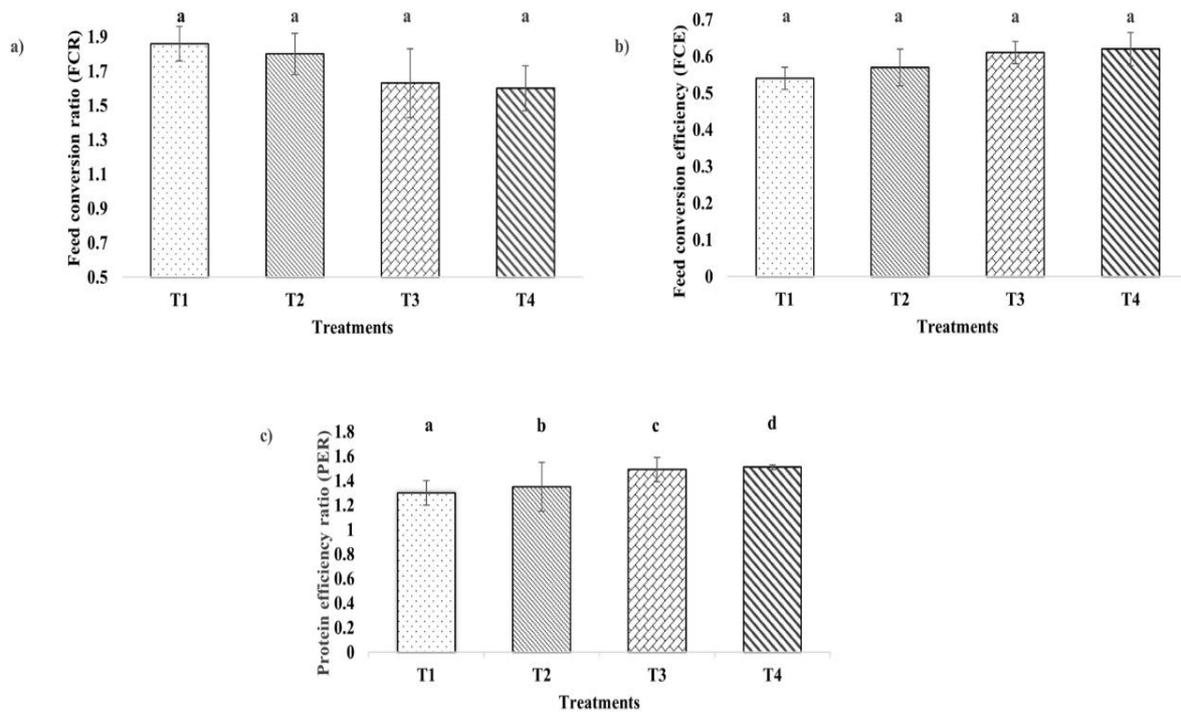


Figure 1. Feed conversion ratio (a), feed conversion efficiency (b) and protein efficiency ratio (c) of *H. fossilis* fed with control (T₁) and probiotic supplemented treatment (T₂, T₃ and T₄) diets for 62 days. Data represent the mean \pm SD and the means with the same or different letters are not significantly ($P > 0.05$) or are significantly ($P < 0.05$) different, respectively.

Findings of this study demonstrated that dietary supplementation of “Navio plus” has positive effects on *H. fossilis* growth and FUPs modulation. Most importantly, more than company recommended dose of this probiotic is also helpful to increase *H. fossilis* production. Farther research is needed to identify its cost-benefit analysis and efficiency of catfish infectious diseases protection. Lastly, results of this study must be cross checked in earthen ponds before application in the large scale commercial aquaculture operations.

Ethical perspective and conflicts of interest

This commercial probiotic “Navio plus” is used for aquaculture purpose and has no ethical issue to be mentioned. The authors have no conflicts of interest.

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