J Bangladesh Agril Univ 18(3): 779–787, 2020 https://doi.org/10.5455/JBAU.107265

ISSN 1810-3030 (Print) 2408-8684 (Online)

Journal of Bangladesh Agricultural University

Journal home page: http://baures.bau.edu.bd/jbau

Effects of Dietary Multi-Strain Probiotics on Growth, Whole Body Composition and Stress Resistance of the Gangetic Catfish (*Mystus Cavasius*) Fry

Tanwi Dey[1]✉, Shakil Akhtar[2], Md. Moshiur Rahman[3], Md. Sakhawat Hossain[1]

[1]Department of Aquaculture, Faculty of Fisheries, Sylhet Agricultural University, Sylhet 3100, Bangladesh
[2]Department of Fisheries Technology and Quality Control, Faculty of Fisheries, Sylhet Agricultural University, Sylhet 3100, Bangladesh
[3]Department of Fish Health Management, Faculty of Fisheries, Sylhet Agricultural University, Sylhet 3100, Bangladesh

ARTICLE INFO

Article history
Received: 15 May 2020
Accepted: 01 Jul 2020
Published online: 10 Jul 2020

Keywords
Probiotics,
Growth,
Feed utilization,
Stress resistance,
Gangetic catfish (gulsha)

Correspondence
Tanwi Dey
✉: tanwi@sau.ac.bd

ABSTRACT

The present study was designed to evaluate the effects of dietary probiotics on growth, feed utilization, body composition, and stress resistance of the Gangetic catfish, *Mystus cavasius* fry. A commercially formulated feed for catfish (ACI Company Ltd.) was used as a basal diet. Three different levels (0.2, 0.4, and 0.8%) of commercial multi-strain probiotic NavioPlus was added to the basal diet to formulate three experimental diets (PB 0.2, PB0.4, PB0.8) and the basal diet was considered as control (PB0). Each diet was randomly allocated to triplicate groups of fish (mean initial weight 0.32±0.0003 g for 56 days. In general, dietary probiotics supplementations tended to improve growth performances (final body weight, percentage (%) weight gain and specific growth rate, SGR), in which significantly highest performances were found in diet group PB-0.2 followed by diet groups PB-0.4 and PB-0.8. Probiotics supplementation resulting in increased feed conversion efficiency (FCE) and protein efficiency ratio (PER) and the highest value ($P<0.05$) obtained in diet group PB-0.4. The control group showed significantly lower growth and feed utilization performances. Except for lipid content, the body proximate compositions were not significantly influenced ($P > 0.05$) by the dietary probiotics supplementation. However, the fishes fed diet PB-0.2 and PB-0 had significantly higher and lower lipid content, respectively. In terms of salinity stress resistance, *M. cavasius* fry fed probiotic supplemented diet showed increased LT_{50} compared to the supplementation free control group. Significantly higher LT_{50} observed in diet group PB-0.4 followed by PB-0.8. The quadratic regression analysis of % weight gain, FCE and, LT_{50} revealed that the optimum level of dietary multi-strain probiotics supplementation ranged between 0.45-0.54% for *M. cavasius* fry, which is also in-line with the most of the measured performance parameters of fish under present experimental condition.

Introduction

The Gangetic catfish, *Mystus cavasius* (gulsha) is a promising species for both monoculture and polyculture practices by virtue of its air-breathing characteristics. It is widely distributed throughout the Indo-Pak Bangladesh sub-continent (Ali *et al*., 2019). In different regions of Bangladesh, day by day culture of this species is getting popular and farmers are culturing this species through semi-intensive/improved semi-intensive systems (Rahman *et al*., 2013). This small indigenous fish species have a good demand as a food fish in the fish markets as it has high nutritional value in terms of protein, micronutrients, vitaminss and minerals. This species also fetches a high price in the market. Due to high demand and price in the market recently this species gained promising attention for commercial culture by the fish farmers (Kohinoor *et al*., 2015). However, the culture of this species is facing several problems; low-quality diets due to insufficient information on the nutritional requirements and disease outbreaks are most important.

The disease is one of the most important limiting factors in aquaculture production as well as in freshwater catfish aquaculture. With the expansion of intensive aquaculture, diseases outbreak has appeared as a more frequent problem in fish culture (Chen *et al*., 2014). Moreover, fish grown in intensive aquaculture systems are often exposed to stressful conditions that have a negative impact on their growth and immunity (Wang *et al*., 2008). For the treatment of various diseases (bacterial, viral, or parasite infections) different

Cite This Article
Dey, T., Akhtar, S., Rahman, M.M., Hossain, M.S. 2020. Effects of Dietary Multi-Strain Probiotics on Growth, Whole Body Composition and Stress Resistance of the Gangetic Catfish (*Mystus Cavasius*) Fry. *Journal of Bangladesh Agricultural University*, 18(3): 779–787. https://doi.org/10.5455/JBAU.107265

types of drugs and chemicals are often used in the aquaculture system, which has several drawbacks. The application of antibiotics and chemotherapeutics to control diseases has been criticized due to the spread of drug-resistant pathogens, suppression of aquatic animal's immune system, and rising environmental hazards (Allameh *et al*., 2016; Brogden *et al*., 2014). In these circumstances, dietary supplementation of health-promoting functional compounds (probiotics, prebiotics, nucleotides, etc.) has received an increasingly high level of interest as an effective alternative for prophylactic treatment against disease outbreaks in semi-intensive and intensive aquaculture (Dawood *et al*., 2015, 2016; Hossain *et al*., 2016a; Hossain *et al*., 2019).

Probiotics have been defined as live micro-organisms that impart positive effects on a host animal by improving the microflora of its gastrointestinal tract (Fuller, 1989). Probiotics are investigated as single-strain and multi-strain products. In the market, however, there is an increasing tendency to work with multi-strain probiotics, in particular, products with a high number of different strains. There are some thoughts behind this: more strains imply more chances of success; it can mean a broader spectrum of efficacy, and there is often the hope that there are at least additive effects and, potentially, even synergistic effects (Ouwehand *et al*., 2018). Although probiotics offer a promising alternative to the use of chemicals and antibiotics in aquatic animals (Bandyopadhyay *et al*., 2015), Recently, several studies have demonstrated that probiotics can improve growth performance, feed utilization, digestibility of dietary ingredients, disease resistance and immunostimulation of aquatic animals (Ferguson *et al.*, 2010; Xing *et al*., 2013; Dawood *et al*., 2015, 2016). Giannenas *et al*. (2015) reported an increased growth performance and health status of rainbow trout fish with the inclusion of multi-strain probiotic containing *Bacillus subtilis, Enterococcus faecium, Pediococcus acidilactici* and, *Lactobacillus* into a commercial type diet which further modulated intestinal microbial communities and positively influenced non-specific immune response. In Bangladesh, although probiotics have been successfully used in shrimp culture (Hossain *et al*., 2013). However, very limited information is available regarding the probiotic's effects on commercially cultured fish species. Recently, Ali *et al*. (2018) and Banu *et al*. (2020) evaluated single strain probiotics in the diets of Gangetic catfish fry and found positive effects of those individual probiotics on growth and feed utilization performances and disease resistance of Gangetic catfish fry. To the best of our knowledge no information is available on the effects of the multi-strain probiotics on the growth, feed utilization, and stress resistance capacity of Gangetic catfish fry. So, in the current study, the impact of a commercial multi-strain probiotic on growth, feed utilization, body proximate compositions, and stress resistance of Gangetic catfish were evaluated.

Methodology

Test fish and experimental design

The experiment was conducted in the post-graduate laboratory of Aquaculture, Faculty of Fisheries, Sylhet Agricultural University, Bangladesh for 56 days. Gangetic catfish fry was collected from a private hatchery in Mymensingh, Bangladesh, and transported by oxygenated polythene bags. Afterward, fish were acclimated in 500 L plastic tank with laboratory facilities for one week with continuous aeration and fed a commercial catfish diet containing 43% protein (ACI company Ltd., Table 1) at the rate of 15% body weight thrice in a day during this period. After acclimatization homogenous sized fry was sorted. Fifty fish, having a mean initial body weight of approximately 0.32±0.0003 g, were randomly allocated into each twelve glass aquaria following Completely Randomized Design (CRD). Four treatments with three replicates for each were used for the experiment. The feeding trial was carried out in twelve glass aquaria of 150 L capacity (filled with 100 L of water). Underground water stored in a reservoir tank was used as a source of freshwater for the aquaria. Each aquarium was equipped with continuous aeration and filtration. Water quality parameters such as temperature, dissolved oxygen, pH, and salinity were measured daily. The behavior of Gangetic catfish was also regularly overlooked especially after feeding to observe their conditions such as movement, infection, colorations, and diseases.

Preparation of experimental diet

Table 1 summarizes the chemical composition of a commercial catfish diet (ACI Company Ltd.) used as the basal diet in this study. The basal diet was grounded with an electric blender (Myako, India) to make it powder form. Afterward, four experimental diets were prepared by supplementing incremental levels of a multi-strain commercial dietary probiotic (Navio plus; Biovac, Thailand) to the basal diet at a level of 0% (P-B0, control), 0.2% (PB-0.2), 0.4% (P-B0.4) and 0.8% (PB-0.8), respectively. "NavioPlus" is the mixture of the following probiotic bacterial and yeast strain: *Bacillus subtilis, B. licheniformis, B. megaterium, Lactobacillus acidophilis, L. plantarum, Saccharomyces cerevisiae*. Initially, the required amount of probiotics was thoroughly mixed by hands 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 (0.5-1.0 mm) to prepare pellets, which were then sun-dried for 5 to 6 hrs. Afterward, they were packed in airtight polythene bags and stored in the refrigerator (4ºC) until fed.

Table 1. Feed formulation and proximate composition (% dry matter basis) of the basal diets.

Ingredients	Composition (%)
Fishmeal	50
Soybean meal	10
Full fat soybean	10
Mustard oil cake	10
Broken wheat	15
Rice bran	4
Vitamin and mineral mixture	1
Total	100
Proximate composition	
Moisture (max.)	11
Protein (min)	43
Lipid (min.)	8
Carbohydrate (max.)	20
Fibre (max.)	3.0
Ash (max.)	10
Calcium (max.)	2
Phosphorous (min.)	1

*According to company information (ACI Co. Ltd.)

Feeding protocol

The experimental diets were fed to fish at 15% of body weight in 1st two weeks of the rearing period which was gradually reduced to 10%, 8%, and 5% every two consecutive weeks, respectively. Daily ration size was divided into three equal feedings (morning at 7:00 a.m., midday at 1:00 p.m. and evening at 7:00 p.m.). Uneaten feed was removed through siphoning after one hour of feeding and 50% water renewal was done at every two days interval to keep the aquatic environment suitable for fish.

Sampling and evaluation of performance parameters

Sampling was performed fortnightly to determine the growth of Gangetic catfish and to adjust the feeding rate. Fish weight was measured with digital balance (CAMRY digital electrical balance, Model EK 3052, Bangladesh). At the end of the 56 days feeding trial, growth and feed utilization parameters of Gangetic catfish *i.e.* % of weight gain, specific growth rate (SGR), feed conversion efficiency (FCE), protein efficiency ratio (PER) and survival (%) were calculated by following equations:

Weight gain = Mean final weight – Mean initial weight.
Percent weight gain (%) = (final weight−initial weight) ×100/initial weight

SGR (%/day) = {Ln (final body weight)-Ln (initial body weight) x 100}/Cultured period (days)

FCE = Wet weight gain of fish (g) / Total amount of dry feed consumed (g)

PER = Weight gain (g)/Protein consumed (g)

Survival rate (%) = (Final number of fish / Initial number of fish) × 100

Saline water stress tolerance test

At the end of the growth trial, a stress tolerance test was conducted to determine the lethal time of 50% mortality (LT_{50}) in saline water containing commercial table salt, NaCl (Dawood *et al*., 2016). After the feeding trial, 8 fish from each rearing aquarium (total 24 fish per treatment) were randomly selected and transferred into 150 L rectangular glass aquarium with 30 L prepared saline water (35 ppt.) which was aerated for 24 hrs. The glass aquaria for stress test were equipped with continuous aeration and kept under ambient temperature during the stress tolerance test. The time duration for 50% mortality of Gangetic catfish fry was calculated according to Hossain *et al*. (2016b) as follows: time to death (min) of fry was converted to log10 values. Immediate after transferring into the saline water the survival rate was 100% (log value was log10 100 = 2) and the calculation was conducted every 5 minutes. The values of log survival rate were plotted against every minute to determine the time needed for 50% mortality of fish fries. The equation is as follows:
Y = aX+ b where Y = log10 (survival) and
X = time for individual fry death (min).
LT_{50} (X) was obtained when Y = 1.7 since log10 (50) = 1.7. Each value obtained from the equation compared statistically.

Proximate composition analysis

The proximate composition as moisture, ash, crude protein, and lipid of fish whole-body was determined in the laboratory of Aquaculture, Faculty of Fisheries, Sylhet Agricultural University by the conventional method of AOAC (2000) with minor modification. The moisture was determined by drying the sample at 105°C to constant weight. The ash was analyzed by combustion at 550°C for 12 h. The crude protein content was determined by measuring the nitrogen content (N×6.25) using the Kjeldahl method with a VELP Scientifica System (DK-8 Series Kjeldahl Digestion Units, Distilling unit UDK139, and Titration unit; Titrette® digital bottle-top burette, Sigma Aldrich). A ground joint Soxhlet apparatus was used for the determination of lipid content.

Statistical analyses

Statistical analysis was performed using a one-way analysis of variance (ANOVA) and Duncan's Multiple Range Test to determine differences between treatments means at significance rate of $P<0.05$. The standard deviation of treatment means was also estimated. Other statistical analyses were carried out using Statistical Package for Social Science (SPSS) version 23. Percentage (%) of WG, FCE, and LT_{50} was subjected to quadratic regression analysis with dietary probiotic supplementation level.

Results

Water quality parameters

The results of different water quality parameters such as temperature, dissolved oxygen, pH, and salinity are summarized in Table 2. Although there were slight variations among different water quality parameters, no significant difference was found among the treatments. The ranges of water quality parameters were temperature 22-24 °C, dissolve oxygen level 5.0-5.5, and pH 6.7 - 7.2.

Table 2: Water quality parameters for 56 days experiment*

Water Quality Parameters	Measurement with SD
Temperature (^{O}C)	19.33±2.52
Dissolved oxygen (mg L^{-1})	5.37±0.21
pH	6.97±0.23
Salinity (ppt.)	0

*Values are means ± SD of triplicate groups.

Growth performance and feed utilization

The growth performance and feed utilization of fish in terms of final weight, % weight gain, SGR (% day^{-1}), feed intake, FCE, PER, and survival are shown in Table 3. Significantly higher final weight and % weight gain observed in the PB-0.2 diet group and it was not significantly different from the PB-0.4 diet group followed by the PB-0.8 diet group. The control group (PB-0.0) showed significantly lower final weight and % weight gain. SGR was not significantly influenced by dietary probiotic supplementations. Feed intake was significantly higher in the control diet group but was not significantly different from the PB-0.2 diet group. PB-0.4 and PB-0.8 groups showed significantly lower feed intake. Probiotics supplementation significantly increases the survival rate of fish and it was significantly higher in the PB-0.4 diet group followed by PB-0.8 and PB-0.2 diet groups. The control group showed a significantly lower survival rate. FCE and PER were significantly influenced by probiotics supplementation and significantly higher FCE and PER values obtained in PB-04 supplemented group which is not significantly different from other supplemented groups. The control group showed significantly lower FCE and PER.

Body proximate compositions

The whole-body proximate composition (% fresh matter basis) of Gangetic catfish at the start and end of the experiment is presented in Table 4. The final carcass moisture content ranged between 79.73 and 80.62% with higher and lower moisture contents was in diet group PB-0.0 and PB-0.2, respectively. There was no significant difference between the whole-body protein content of fish in different treatments which ranged between 13.21 and 13.36%. Body lipid significantly influenced by probiotics supplementation and significantly higher whole-body lipid observed in the PB-0.2 diet group (3.87%). The control group (PB-0.0) showed significantly lower whole-body lipid content (3.13%). The ash content varied from 2.33 to 2.57% with treatments PB-0.2 and PB-0.4 showing the highest and lowest values respectively. However, ash content was not significantly influenced by probiotics supplementation.

Saline water stress tolerance test

The results of the lethal stress test of LT_{50} against saline water shock were obtained by regression analysis is presented in Figure 1. Supplementation of probiotics improved the saline water stress tolerance of Gangetic catfish. Significantly highest LT_{50} (46.55 min) obtained in the PB-0.4 diet group followed by the PB-0.8 diet group (41.4 min). The control group showed significantly lower LT_{50} value (32.5 min.) and it was not significantly different from the PB-0.2 diet group (36.0 min).

Optimum supplementation of multi-strain probiotics

The optimum supplementation level of multi-strain probiotics for % weight gain, FCE, and LT_{50} estimated by quadratic regression analysis (Fig. 2a,b,c). Based on % weight gain, the optimal supplementation level of multi-strain probiotics was estimated to be 0.45% in the diet. On the other hand, 0.54% level in the diet was estimated as optimal based on FCE and LT_{50}.

Discussion

Growth rate, feed efficiency and feed consumption of fish normally governed by a few environmental factors (Fry, 1971; Brett, 1979). Environmental parameter exerts an immense influence on the maintenance of a healthy aquatic environment and production of food organisms. The water quality parameters such as temperature, pH, and dissolved oxygen were recorded daily during the experimental period (Table 2). The water quality parameters measured in the different aquaria in the present study were found to be more or less similar and were within the acceptable range for catfish culture (Giri *et al*., 2010; Paul *et al*., 2012). No negative effects on water quality parameters such as dissolve oxygen, temperature, and pH in the current study observed due to probiotics supplementation in aquafeed which also resembled with the findings of Wang & Zirong (2006); they reported that no important effects in water quality parameters were observed when common carp was fed with probiotic photosynthetic bacteria and *Bacillus* sp.

Table 3: Growth performance and feed utilization of Gangetic catfih fry fed with experimental diets for 56 days*.

Parameters	Diet groups			
	PB-0	PB-0.2	PB-0.4	PB-0.8
Initial weight	0.32±0.0003	0.32±0.0001	0.32±0.001	0.32±0.0002
Final weight	1.53±0.04a	1.82±0.05b	1.81±0.11b	1.67±0.03ab
Weight gain (%)	369.67±11.19a	461.34±15.11b	455.58±33.27b	414.29±9.68ab
SGR	2.76±0.04	3.08±0.05	3.06±0.11	2.92±0.03
Survival	65.0±2.29a	81.66±4.41ab	95.0±0.00b	91.67±6.01b
Feed intake	6.15±0.13b	5.30±0.15ab	4.58±0.17a	4.56±0.32a
FCE	0.20±0.008a	0.28±0.01b	0.32±0.01b	0.30±0.03b
PER	0.55±0.02a	0.81±0.04b	0.91±0.03b	0.84±0.07b

*Values are means ± SD of triplicate groups. Values in the same row having same superscripts are not significantly different (P>0.05). Specific growth rate, SGR; Feed conversion efficiency, FCE; Protein efficiency ratio, PER.

Table 4. Carcass composition of Gangetic catfish fry fed with different levels of probiotics for 56 days

Parameters	Initial	Diet groups			
		PB0	PB0.2	PB0.4	PB0.8
Moisture (%)	81.12	80.62*±0.31	79.73±0.20	80.05±.23	80.22±.25
Protein (%)	12.77	13.25±0.10	13.36±0.14	13.31±0.14	13.21±0.12
Lipid (%)	1.58	3.13^{b}±0.32	3.87^{a}±0.14	3.52^{b}±0.18	3.50^{b}±0.18
Ash (%)	2.24	2.44±0.10	2.57±0.17	2.33±0.20	2.43±0.23

** Values are mean ± standard deviation. Values in the same row having same superscripts are not significantly different (P>0.05).

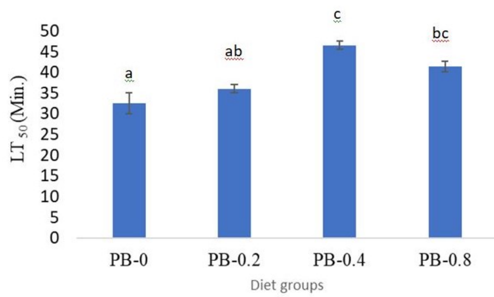
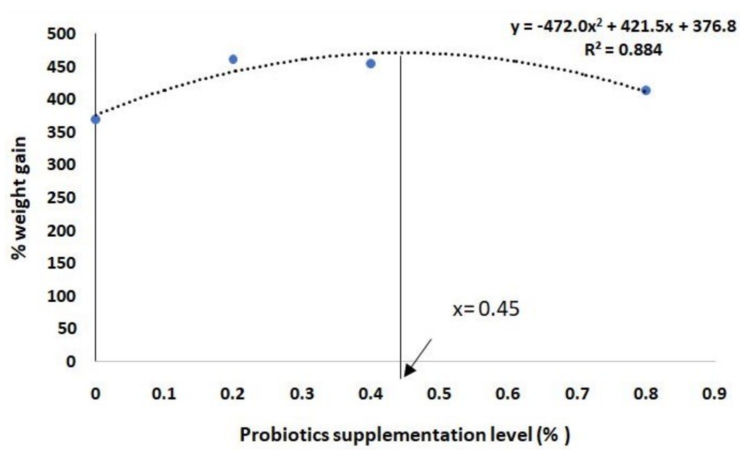


Figure 1. LT50 (min) calculated from the lethal time of Gangetic catfish exposed to saline water. Data are expressed as mean ± standard deviation from triplicate groups. Bars having same letters are not significantly different at 5% level of significance.

2(a)



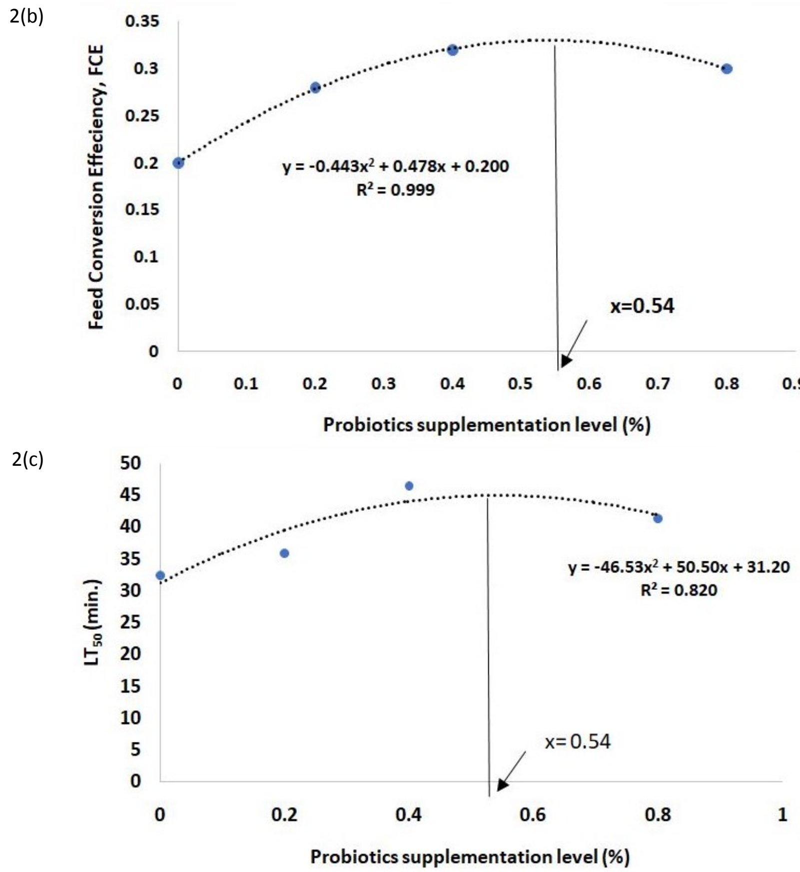


Figure 2. Quadratic regression analyses of (a) weight gain, (b) feed conversion efficiency, and (c) LT50 for Gangetic catfish fry fed diet supplemented with graded level of multi-strain probiotics

The obtained performance data showed that supplementation of multi-strain probiotics at 0.2, 0.4, and 0.8% in the diets improved the growth, and feed utilization performance of Gangetic catfish fry. Similar findings were reported in previous studies (Hoseinifar *et al*., 2011; Xing *et al*. 2013; Dawood *et al*., 2015, 2016; Banu *et al*., 2020). This increased growth and feed utilization performances of Gangetic catfish might be due to the increased digestive enzyme activity, elevated health status, and stimulation of gastric development and /or enzymatic secretion (Suzer *et al*., 2008; Khonyoung and Yamauchi, 2012) in probiotics supplemented groups. Although in the current study digestibility as well as the amount of enzyme secretion was not determined. However, studies on several fish species reported that dietary supplementation of probiotic form yeasts and bacteria could increase digestive enzyme activity in sea bass (*Dicentrarchus labrax*) (Tovar *et al*., 2002), rainbow trout (*Oncorhynchus mykiss*) (Adel *et al*., 2017), abalone (*Haliotis midae*) (Macey and Coyne, 2005), sea cucumber (Yang *et al*., 2014; Wang *et al*., 2015) and red sea bream (Dawood *et al*., 2015). In the current study growth and feed utilization performances showed a dose-response relationship and after administrating a certain dose (>.0.4%) of probiotics growth and feed utilization tended to decrease. Like the current study similar dose-response relationships were also observed for other fish species fed with different levels of probiotic supplemented diets. Dawood *et al*. (2015) reported reduced growth performance when red sea bream was fed heat-killed *Lactobacillus plantarum* at a high dose (2000ppm). The report of Ghosh *et al*. (2008) also showed that the use of higher concentration of probiotic did not always lead to better growth performances of ornamental fish growth which strongly substantiate with the current findings. Banu *et al*. (2020) also reported reduced growth and feed utilization performance in Gangetic catfish when fed with a high level of yeast probiotics (0.15%) which suggested these adverse effects might arose due to the adverse effects on metabolism and other physiological functions in fish under a higher dose. Therefore, it is suggested that optimum supplementation is very important for the better functioning of probiotics in aquafeed.

Endogenous factors such as fish size, sex as well as exogenous factors such as diet composition and culture environment influence the proximate composition of fish (Shearer, 1994). In this experiment carcass composition was influenced by different probiotics levels in feeds. There was a marked increase in the lipid content of fish fed probiotics compared to the initial lipid content of fish (Table 4). Mohapatra *et al.* (2012) reported high lipase secretion by most probiotics which influences the assimilation of fatty acids that influences immunity in fish. The carcass lipid content was directly influenced by the dietary moisture content. An inverse relationship of carcass moisture and lipid content was observed as reported earlier in fish (Andrews and Stickney, 1972; Garling and Wilson, 1976; Jauncey, 1982). However, there was no significant variation in carcass protein content of fish due to different levels of probiotics feeding.

Stress is one of the emerging factors in aquaculture activities, which may affect hormonal secretion rates, intermediary metabolism, immunity, and nutrient utilization (Hossain *et al.*, 2016a,b). Dietary administration of different nutritional or immunostimulant elements such as probiotics, prebiotics, nucleotides, and other functional supplements 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, 2017, 2018). Unfortunately, there were no similar studies available on the effects of dietary probiotics on salinity stress responses of Gangetic catfish fry to compare the result of the present study. In the present study, Gangetic catfish fed probiotic supplemented diets showed increased salinity stress resistance after exposure to a high salinity stress test. In the present study, the highest LT_{50} value obtained in diet group PB-0.4 (46.5 min) followed by PB-0.8 diet group. On the other hand, supplementation free control group, and the lower dose of probiotics supplemented diet group (PB-0.2) showed the significantly lower values of LT_{50} indicated a lower saline water stress tolerance of the Gangetic catfish. Many authors reported high salinity stress resistance after feeding on probiotics supplemented diet (Hoseinifar *et al.*, 2013b, Soleimani *et al.*, 2012). However, overall increased LT_{50} values under probiotic supplemented diets indicate the healthy status of Gangetic catfish (Hossain *et al.*, 2016b; Yokoyama *et al.*, 2005).

The significantly influenced % weight gain, FCE, and LT_{50} were used for regression analysis to estimate the optimum supplementation level of multi-strain probiotics in the present study. Based on the quadratic regression analysis of % weight gain the optimum level of dietary multi-strain probiotics might be 0.45% and considering FCE and LT_{50}, the optimum level might be 0.54%. Results of the present study indicated that optimum supplementation of multi-strain probiotics in Gangetic catfish fry for enhanced feed utilization and stress tolerance were a little bit higher than for growth performance.

Conclusion

Based on the present experimental condition, it can be concluded that the optimum level of multi-strain probiotics supplementation ranged between 0.45 to 0.54 % in the diets to positively influence the growth response, feed utilization and salinity stress tolerance of Gangetic catfish fry.

Acknowledgement

This research work was partially funded by the Sylhet Agricultural University Research System (SARES), Sylhet Agricultural University, Sylhet, Bangladesh provided to Dr. Md. Sakhawat Hossain

Conflict of Interests

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

References

Adel, M., Lazado, C.C., Safari, R., Yeganeh, S., and Zorriehzahra, M.J., 2017. Aqualase®, a yeast-based in-feed probiotic, modulates intestinal microbiota, immunity, and growth of rainbow trout *Oncorhynchus mykiss*. Aquaculture Research, 48: 1815-1826. https://doi.org/10.1111/are.13019

Ali, M.Z., Paul, M., Jana, P., Rahman, M.K. and Mahmud, Y., 2018. Evaluation of Selected Dietary Probiotics on Growth Performance, Nutrient Utilization, and Body Carcass Composition of *Mystus Cavasius* (Hamilton, 1822) Fry. International Journal of Current Microbiology and Applied Sciences, 7(8): 3328-3339. https://doi.org/10.20546/ijcmas.2018.708.356

Allameh, S.K., Yusoff, F.M., Ringø, E., Daud, H.M., Saad, C.R., and Ideris, A., 2016. Effects of dietary mono-and multi probiotic strains on growth performance, gut bacteria, and body composition of Javanese carp (*Puntius gonionotus,* Bleeker 1850). Aquaculture Nutrition, 22(2): 367-373. https://doi.org/10.1111/anu.12265

Andrews, J.W., and Stickney, R.R., 1972. Interaction of feeding rates, and environmental temperature on growth, food conversion, and body composition of channel catfish. Transactions of the American Fisheries Society, 101(1): 94-97. https://doi.org/10.1577/1548-8659(1972)101<94:IOFRAE>2.0.CO;2

AOAC, 2000. Official methods of Analysis. 17th ed. Association of Official Analytical Chemist, Washington DC. 2200 pp.

Bandyopadhyay, P., Mishra, S., Sarkar, B., Swain, S.K., Pal, A., Tripathy, P.P., and Ojha, S.K., 2015. Dietary Saccharomyces cerevisiae Boosts Growth and Immunity of IMC *Labeo rohita (Ham.*) Juveniles. Indian Journal of Microbiology, 55: 81-87. https://doi.org/10.1007/s12088-014-0500-x

Banu, M.R., Akter, S., Islam, M.R., Mondol, M.N., and Hossain, M.A., 2020. Probiotic yeast enhanced growth performance and disease resistance in freshwater catfish gulsa tengra, *Mystus cavasius*. Aquaculture Report, 16: 100-237. https://doi.org/10.1016/j.aqrep.2019.100237

Beck, B.R., Kim, D., Jeon, J., Lee, S. M., Kim, H. K., Kim, O. J., and Song, S. K., 2015. The effects of combined dietary probiotics Lactococcus lactis BFE920 and Lactobacillus plantarum FGL0001 on innate immunity and disease resistance in olive flounder (*Paralichthys olivaceus*). Fish and Shellfish Immunology 42(1):177-183. https://doi.org/10.1016/j.fsi.2014.10.035

Brett, J.R., 1979. Environmental factors and growth. In: Fish Physiology. W. S. Hoar; D. J. Randall and J. R. Brett (eds.) Vol 6. Academic Press, New York. Pp. 599-667. https://doi.org/10.1016/S1546-5098(08)60033-3

Brogden, G., Krimmling, T., Adamek, M., Naim, H.Y., Steinhagen, D., and Von Kockritz-Blickwede, M., 2014. The effect of β-glucan on formation and functionality of neutrophil extracellular traps in carp (*Cyprinus carpio L*.). Developmental & Comparative Immunology, 44: 280-285. https://doi.org/10.1016/j.dci.2014.01.003

Chen, Y., Zhu, X., Yang, Y., Han, D., Jin, J., and Xie, S., 2014. Effect of dietary chitosan on growth performance, haematology, immune response, intestine morphology, intestine microbiota, and disease resistance in gibel carp (*Carassius auratus gibelio*). Aquaculture Nutrition, 20: 532-546. https://doi.org/10.1111/anu.12106

Dawood, M.A.O., Koshio, S., Ishikawa, M., and Yokoyama, S., 2016. Immune responses and stress resistance in red sea bream, *Pagrus major*, after oral administration of heat-killed Lactobacillus plantarum and vitamin C. Fish and Shellfish Immunology, 54: 266-275. https://doi.org/10.1016/j.fsi.2016.04.017

Dawood, M.A.O., Koshio, S., Ishikawa, M., and Yokoyama S., 2015. Effects of heat-killed *Lactobacillus plantarum* (LP20) supplemental diets on growth performance, stress resistance, and immune response of red sea bream, *Pagrus major.* Aquaculture, 442: 29-36. https://doi.org/10.1016/j.aquaculture.2015.02.005

Ferguson, R.M.W., Merrifield, D.L., Harper, G.M., Rawling, M.D., Mustafa, S., Picchietti, S., Balc_azar, J.L., and Davies, S.J., 2010. The effect of *Pediococcus acidilactici* on the gut microbiota and immune status of on-growing red tilapia (*Oreochromis niloticus*). Journal of Applied Microbiology, 109: 851-862. https://doi.org/10.1111/j.1365-2672.2010.04713.x

Fry, F.E. 1971. The effect of environmental factors on the physiology of fish. In: W. S. Hoar; D. R. Randall and J. R. Brett (eds.), Fish physiology. Vol.6, Academic Press, New York. pp 1-87. https://doi.org/10.1016/S1546-5098(08)60146-6

Fuller, R. 1989. A review: probiotics in man and animals. Journal of Applied Bacteriology, 66: 365-378. https://doi.org/10.1111/j.1365-2672.1989.tb05105.x

Garling, D.L. Jr. and Willson, R.P., 1976. Optimum dietary protein to energy ratio for channel catfish fingerlings, *Ictalurus punctatus*, Journal of Nutrition, 106: 1368-1375. https://doi.org/10.1093/jn/106.9.1368

Ghosh S., Sinha A., and Sahu C., 2008. Dietary probiotic supplementation in growth and health of live-bearing ornamental fishes. Aquaculture Nutrition, 14: 289-299. https://doi.org/10.1111/j.1365-2095.2007.00529.x

Giri, S.S., Sahoo, S.K., and Mohanty, S.N., 2010. Replacements of by-catch fish meal with dried chicken viscera meal in extruded feeds: effect on growth, nutrient utilization, and carcass composition of catfish *clarias batrachus* (Linn.) fingerlings. Aquaculture International, 18: 539-544. https://doi.org/10.1007/s10499-009-9265-3

Giannenas, I., Karamaligas, I., Margaroni, M., Pappas, I., Mayer, E., Encarnacao, P. and Karagouni, E., 2015. Effect of dietary incorporation of a multi-strain probiotic on growth performance and health status in rainbow trout (*Oncorhynchus mykiss*). Fish Physiology and Biochemistry, 41(1): 119-128. https://doi.org/10.1007/s10695-014-0010-0

Hoseinifar, S.H., Mirvaghefi, A. and Merrifield, D.L., 2011a. The effects of dietary inactive brewer's yeast Saccharomyces cerevisiae var. ellipsoideus on the growth, physiological responses, and gut microbiota of juvenile beluga (*Huso huso*). Aquaculture, 318 (1-2): 90-94. https://doi.org/10.1016/j.aquaculture.2011.04.043

Hoseinifar, S.H., Khalili, M., Khoshbavar, H.R., and Esteban, M. 2013b. Dietary galactooligosaccharide affects intestinal microbiota, stress resistance, and performance of Caspian roach (*Rutilus rutilus*) fry. Fish Shellfish Immunology, 35:1416-20. https://doi.org/10.1016/j.fsi.2013.08.007

Hossain, M.S., Koshio S., Ishikawa M., Yokoyama S. and Sony, N. M., 2016c. Dietary effects of adenosine monophosphate to enhance growth, digestibility, innate immune responses, and stress resistance of juvenile red sea bream, *Pagrus major*. Fish and Shellfish Immunology, 56: 523-533. https://doi.org/10.1016/j.fsi.2016.08.009

Hossain, M.S., Koshio S., Ishikawa M., Yokoyama S., Sony, N.M., Dossou, S., and Wang, W., 2018. Influence of dietary inosine and vitamin C supplementation on growth, blood chemistry, oxidative stress, innate and adaptive immune responses of red sea bream, *Pagrus major* juvenile. Fish and Shellfish Immunology, 82: 92-100. https://doi.org/10.1016/j.fsi.2018.08.014

Hossain, M.S., Koshio S., Ishikawa M., Yokoyama S., Sony, N. M. and Fujieda, T., 2017. Nucleoside by-product dietary supplementation influences blood chemistry, immune response, oxidative stress resistance, and intestinal morphology of juvenile amberjack, *Seriola dumerili*. Aquaculture Nutrition*,* 23(6): 1390-1400. https://doi.org/10.1111/anu.12514

Hossain, M.S., Koshio, S., Ishikawa M., Yokoyama S., Sony N.M., Ono, S. and Fujieda T., 2016b. Comparison of the effects of inosine and inosine monophosphate on growth, immune response, stress resistance, and gut morphology of juvenile red sea bream, *Pagrus major*. Aquaculture, 458: 64-74. https://doi.org/10.1111/anu.12514

Hossain, M.S., Koshio S, Ishikawa M, Yokoyama, S. and Sony, N.M., 2016a. Dietary nucleotide administration influences growth, immune responses, and oxidative stress resistance of juvenile red sea bream (*Pagrus major*). Aquaculture, 455: 41-49. https://doi.org/10.1016/j.aquaculture.2016.01.008

Hossain, M.I., Kamal, M.M., Mannan, M.A., Bhuyain, M.A.B. and Hossain, M.I., 2013. Effects of Probiotics on Growth and Survival of Shrimp (*Penaeus monodon*) in Coastal Pond at Khulna. Bangladesh Journal of Scientific Research, 5 (2): 363-370. https://doi.org/10.3329/jsr.v5i2.11815

Hossain, M.S., Koshio, S., and Kestemont, P., 2019. Recent advances in nucleotide nutrition research in aquaculture: a review. Reviews in Aquaculture, 1-26. https://doi.org/10.1111/raq.12370

Jauncey, K., 1982. The effect of varying dietary protein levels on the growth, food conversion, protein utilization, and body composition of juvenile tilapia (*Sarotherodon mossambicus*). Aquaculture*,* 27, 43-54. https://doi.org/10.1016/0044-8486(82)90108-9

Khonyoung, D., and Yamauchi, K., 2012. Effects of heat-killed Lactobacillus plantarum L-137 on the morphology of intestinal villi and epithelial cells in broiler chickens. Journal of Applied Animal Research, 40 (2): 140-147. https://doi.org/10.1080/09712119.2011.640208

Kohinoor, A.H.M., and Rahman, M., 2015. Growth and production performances of threatened small indigenous fish Gulsha (*Mystus cavasius*) in the cage system in the River Brahmaputra, Mymensingh, Bangladesh. International Journal of Fisheries and Aquatic Studies, 2(5): 180-183.

Macey, B.M., and Coyne, V.E., 2005. Improved growth rate and disease resistance in farmed *Haliotis midae* through probiotic treatment. Aquaculture, 245: 249-261. https://doi.org/10.1016/j.aquaculture.2004.11.031

Mohapatra, S., Chakraborty, T., and Prusty, A. K., 2012. Use of different microbial probiotics in the diet of rohu, *Labeo rohita* fingerlings: effects on growth, nutrient digestibility and retention, digestive enzyme activities, and intestinal microflora. Aquaculture Nutrition, 18:1-11. https://doi.org/10.1111/j.1365-2095.2011.00866.x

Nayak, S.K., Swain P., and Mukherjee, S.C., 2007. Effect of dietary supplementation of probiotics and vitamin C on the immune response of Indian major carp, *Labeo rohita* (Ham.). Fish and Shellfish Immunology, 23(4): 892-896. https://doi.org/10.1016/j.fsi.2007.02.008

Ouwehand, A.C., Invernici, M.M., Furlaneto, F.A.C., and Messora, M.R., 2018. Effectiveness of Multi-strain Versus Single-strain Probiotics, Journal of Clinical Gastroenterology, 52: 35-40. https://doi.org/10.1097/MCG.0000000000001052

Paul, B.N., Das, S., Datta, A.K., Giri, S.S. and Mohanty1, S.N., 2012. Effect of Dietary Protein Levels on Growth of *Ompok pabda* (Siluridae) Fry. Animal Nutrition and Feed Technology, 12: 241-246.

Rahman, M.A., Zaher, M., Azimuddin, K.M., Yeasmine, S., Khan, M.M., and Arshad, A., 2013. Stocking density effects on growth and production of the threatened silurid catfish, *Mystus cavasius* (Hamilton) fingerlings in nursery ponds. Aquaculture Research, 44: 1132-1139. https://doi.org/10.1111/j.1365-2109.2012.03148.x

Soleimani, N., Hoseinifar, S.H., Merrifield, D.L., Barati, M., and Abadi, Z.H., 2012. Dietary supplementation of fructooligosaccharide (FOS) improves the innate immune response, stress resistance, digestive enzyme activities, and growth performance of Caspian roach (Rutilus rutilus) fry. Fish Shellfish Immunology, 32: 316-321. https://doi.org/10.1016/j.fsi.2011.11.023

Shearer, K.D., 1994. Factors affecting the proximate composition of cultured fishes with emphasis on salmonids. Aquaculture, 119: 63-88. https://doi.org/10.1016/0044-8486(94)90444-8

Suzer, C., Coban, D., Kamaci, H.O., Saka, S., Firat, K., Otgucuoglu, O., and Kucuksari, H., 2008. *Lactobacillus* spp. bacteria as probiotics in gilthead sea bream (*Sparus aurata*, *L.*) larvae: effects on growth performance and digestive enzyme activities. Aquaculture, 280: 140-145. https://doi.org/10.1016/j.aquaculture.2008.04.020

Tovar, D., Zambonino, J., Cahu, C., Gatesoupe, F.J., Vazquez-Juárez, R., and Lesel, R., 2002. Effect of live yeast incorporation in compound diet on digestive enzyme activity in sea bass (*Dicentrarchus labrax*) larvae. Aquaculture, 204 (1-2): 113-123. https://doi.org/10.1016/S0044-8486(01)00650-0

Wang, Y. and Zirong, X., 2006. Effect of probiotics for common carp (*Cyprinus carpio*) based on growth performance and digestive enzyme activities. Animal Feed Science and Technology, 127(3-4): 283-292. https://doi.org/10.1016/j.anifeedsci.2005.09.003

Wang, J.H., Zhao, L.Q., Liu, F., Wang, H., and Xiao, S., 2015. Effect of potential probiotic *Rhodotorula benthica* D30 on the growth performance, digestive enzyme activity, and immunity in juvenile sea cucumber *Apostichopus japonicus*. Fish Shellfish Immunology, 43: 330-336. https://doi.org/10.1016/j.fsi.2014.12.028

Wang, Y., Li, J., and Lin, J., 2008. Probiotics in aquaculture: challenges and outlook. Aquaculture, 281: 1-4. https://doi.org/10.1016/j.aquaculture.2008.06.002

Xing, C.F., Hu, H.H., Huang, J.B., Fang, H.C., Kai, Y.H., Wu, Y.C., and Chi, S.C., 2013. Diet supplementation of *Pediococcus pentosaceus* in cobia (*Rachycentron canadum*) enhances growth rate, respiratory burst, and resistance against photobacteriosis. Fish Shellfish Immunology, 35: 1122-1128. https://doi.org/10.1016/j.fsi.2013.07.021

Yang, Z.P., Sun, J.M., Xu, Z., Zhang, C.C., and Zhou, Q., 2014. Beneficial effects of *Metschnikowia* sp. C14 on growth and intestinal digestive enzymes of juvenile sea cucumber *Apostichopus japonicus*. Animal Feed Science and Technology, 197: 142-147. https://doi.org/10.1016/j.anifeedsci.2014.07.013

Yokoyama, S., Koshio, S., Takakura, N., Oshida, K., Ishikawa, M., Gallardo-Cigarroa, F.J., and Teshima, S., 2005. Dietary bovine lactoferrin enhances tolerance to high temperature stress in Japanese flounder *Paralichthys olivaceus*. Aquaculture, 249: 367-73. https://doi.org/10.1016/j.aquaculture.2005.03.024

Rahaman, A. 2010. Participation of Rural Youth in Fish Farming Activities MS thesis, Department of Agricultural Extension Education, Bangladesh Agricultural University, Mymensingh, Bangladesh.

Roy, P.,Uddin, M N, Miah, M A M and Akthar, S. 2019. Identification of Problems Faced by the Rural Women in Receiving Services Provided by the BRAC on food and Nutrition: A Case of Rural Bangladesh, Discovery Agriculture, 5: 221-228

Suvedi, M., Ghimire, R. & Kaplowitz, M. (2017). Farmers' Participation in Extension Programs and Technology Adoption in Rural Nepal: A Logistic Regression Analysis, The Journal of Agricultural Education and Extension, 23:4, 351-371, https://doi.org/10.1080/1389224X.2017.1323653

Uddin, M, N, Hasan, M.M. and Miah, M A M. 2018. Effectiveness of Flood Coping Strategies Practiced by the Fish Farmers: An Empirical Study in Bangladesh, Journal of Agroecology and Natural Resource Management 5(2): 82-86

Uddin, M. N.; Shajahan, M.; Miah, M A. M. and Yeasmin, F. 2019. Use of Mobile Phone in Receiving Information on Fish Culture: A Case Study of Farmers in Bangladesh, Discovery, 55(281): 214-219

Younus, M. A. F. 2012. "Autonomous Crop Adaptation Processes to Extreme Floods: A Case Study in Bangladesh." In Water and Climate: Policy Implementation Challenges: Proceedings of the 2nd Practical Responses to Climate Change Conference, edited by K. A. Daniell, 124–131. Barton, A.C.T.: Engineers Australia.

Younus, M., and N. Harvey. 2014. "Economic Consequences of Failed Autonomous Adaptation to Extreme Floods: A Case Study from Bangladesh." Local Economy 29 (1): 20–35. https://doi.org/10.1177/0269094213515175

Zaman, N. 2010. Participation of Rural Women in Homestead Fruit production MS thesis, Department of Agricultural Extension Education, Bangladesh Agricultural University, Mymensingh, Bangladesh.