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Effect of probiotics on growth, feed utilization and whole body proximate composition of pabda (*Ompok pabda*) juvenile

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Abstract
The study was conducted to determine the effects of probiotics on growth, feed utilization and whole body proximate composition of Pabda (*Ompok pabda*) in laboratory condition for a period of 120 days under four treatments with three replication. Treatments were T_1 (basal feed+0% probiotics), T_2 (basal feed+0.2% probiotics), T_3 (basal feed+0.4% probiotics) and T_4 (basal feed+0.8% probiotics). Significantly highest mean final weight was recorded in T_2 (5.81±0.11 g) followed by T_3 (5.29±0.15 g), T_4 (5.24±0.03 g) and T_1 (4.70±0.49 g). The lowest feed conversion ratio (FCR) was obtained in T_2 (1.36±0.04) than that of T_1 (1.86±0.21), T_3 (1.55±0.08) and T_4 (1.55±0.09). Highest SGR value (1.65±0.02) was found in T_2 than that of (1.41±0.12) T_1, (1.54±0.01) T_4 and (1.55±0.04) T_3. Significantly highest PER was in T_2 (2.10±0.07) compared to T_1 (1.52±0.16), T_3 (1.81±0.09) and T_4 (1.83±0.10). Higher survival of Pabda was found in T_2 (94.44±5.55 %) and T_3 (94.44±5.55 %) and lower in T_1 (92.59±8.50 %) and T_4 (92.59±6.41 %). The highest moisture content was observed in T_4 (75.83±0.12 %) followed by T_1 (74.84±0.17%), T_3 (74.46±0.11 %) and T_2 (74.38±0.10 %). The highest ash content was obtained in T_1 (4.25±0.14 %) followed by T_2 (3.84±0.03%), T_4 (3.58±0.13 %) and T_3 (3.33±0.46 %). Significantly highest protein content of Pabda was found in T_3 (16.04±0.13 %) followed by T_2 (15.69±0.10 %), T_4 (15.05±0.09 %), T_1 (14.97±0.08 %) and highest lipid contents of Pabda obtained in T_3 (5.82±0.14 %) followed by T_4 (5.38±0.14 %), T_2 (5.08±0.14 %) and T_1 (4.87±0.09 %). Therefore, results of the study imply that probiotics treated fish showed better growth and feed utilization and 0.2% of probiotics (T_2) showed better growth performance of *Ompok pabda*.

Keywords: Probiotics, pabda, growth, proximate composition

1. Introduction
Ompok pabda is a member of the family siluridae under the order siluriformes which is commonly known “Pabda” or “Pabo”. This small indigenous fish species has a good demand as a food fish in the fish markets as it has high nutritional value. Due to high demand and price in the market recently this species gained promising attention for commercial culture by the fish farmers. However, cultures of this species are facing several problems, among them lack of quality diet development due to insufficient nutritional requirement information’s and diseases are most important.

Disease is one of the most important limiting factors in aquaculture production. For the treatment of various diseases (bacterial, viral or parasite infections) different types of drugs and chemicals are often used in aquaculture system. The excessive use of antibiotics, drugs, pesticides and antiseptics to prevent bacterial diseases and to promote juvenile growth has led to the development of resistant bacterial strains and subsequent potential safety issues [2]. Increase in bacterial resistance to antibiotics has made attention to research into the usage of probiotics, as it can exert benefits on host welfare other than nutritional support [6].

Probiotics are known as feed additives which are defined as live microbes that may serve as dietary supplements to improve the host intestinal microbial balance and growth performance [10]. There are a wide range of microalgae (*Tetraselmis*), yeast (*Debaryomyces*, *Phaffia* and *Saccharomyces*) and gram positive (*Bacillus*, *Lactococcus*, *Micrococcus*, *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Streptococcus* and *Weissella*) and gram negative bacteria (*Aeromonas*, *Alteromonas*, *Photorhodobacterium*, *Pseudomonas* and *Vibrio*) that have been evaluated as a probiotics [10]. The probiotics in aquaculture have been shown to have several modes of action: competitive exclusion of pathogenic bacteria through the production of,

inhibitory compounds improvement of water quality, enhancement of immense response of host species and enhancement of nutrition of host species through the production of inhibitory of supplemental digestive enzymes [7].

Nowadays, probiotics are becoming an integral part of the aquaculture practices to obtain high production [20]. Feeding supplemented with probiotics improve appetite and growth performance of the farmed fish [11]. The need for increased disease resistance, growth of aquatic organisms and feed efficiency has brought about the use of probiotics in aquaculture practices [8]. Proximate analysis is used to estimate the relative amounts of protein, lipid, moisture, ash and carbohydrate in an organism. Fish carcass contains on average 75% water, 16% protein, 6% lipid and 3% ash. In some previous researches it is also reported that supplementation of health promoting functional feed additives affects the whole body proximate composition of fish [9, 12].

With increasing demand for environment friendly aquaculture, the use of probiotics in aquaculture is now widely accepted [10, 13]. But most of the farmers of our country don't know the uses, impacts, doses of these probiotics for commonly cultured Pabda catfish. Therefore, considering the above circumstances the present study was conducted to assess the effects of dietary probiotics supplementation on growth, feed utilization, whole body proximate composition of Pabda.

2. Materials and Methods

2.1 Study area and period

The experiment was conducted in post-graduate laboratory of Aquaculture, Faculty of Fisheries, Sylhet Agricultural University, Sylhet. The experiment was conducted for a period of 120 days from 15 September, 2017 to 13 January, 2018 in 12 aquarium.

2.2 Preparation of experimental diet

A commercial formulated feed for catfish (ACI Company Ltd.) was used as a basal diet. Tables 1, summarize the chemical composition of the basal diet. The basal diet was grounded with an electric blender to make it powder form. Afterward four experimental diets were prepared by supplementing incremental levels of a commercial dietary probiotic (commercial name: Navio plus; Manufactured by: Biovac, Thailand) to the basal diet at 0% (PB0, control), 0.2% (PB0.2), 0.4% (PB0.4) and 0.8% (PB0.8), respectively. Initially required amount of probiotics were thoroughly mixed by hands with the grounded basal diet. After proper mixing, water was added gradually (35–40%) of the dry ingredients. The mixture was then passed through a kitchen type pellet machine with an appropriate diameter (1.2mm) to prepare pellets, which were then sun-dried at ambient temperature (35°C–40°C) for 5 to 6 h and packed in airtight polythene bags and stored at refrigerator until fed.

Table 1: Proximate composition of basal diets

Proximate Composition	Percentage (%)
Moisture (max.)	11
Protein (min)	44
Lipid (min.)	8
Carbohydrate (max.)	21
Fibre (max.)	3.0
Ash (max.)	10
Calcium (max.)	2
Phosphorous (min.)	1

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

2.3 Collection and acclimatization of Pabda fry

Pabda fry were collected from a private hatchery in Mymensingh and were transported in oxygenated polythene bags from the hatchery to University campus. Before stocking fries were acclimatized to the aquarium water gradually for 7days.

2.4 Experimental fish and feeding protocol

After being acclimatized to the laboratory environment, homogenous sized juveniles were sorted. Eighteen fish, having a mean initial body weight of approximately 1.31 g were randomly allocated to each of twelve previously prepared glass aquarium. Fish were fed the experimental diets at 5% of their body weight in first month of their rearing period which was gradually reduced to 4%, 3% and 2% of their body weight in 2nd, 3rd and 4th month, respectively. Daily ration size was divided into three equal feedings. Monthly sampling was carried out using a scope net to monitor the fish growth and adjust the ration size. Behavior of Pabda was also regularly observed especially after feeding, to observe their conditions such as movement, infection, colorations and diseases.

2.5 Estimation of growth and feed utilization of Pabda

Growth parameter such as % of weight gain, specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and survival (%) were calculated by following the equation [17]:

Weight gained = Mean final weight – Mean initial weight

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

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

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

Protein efficiency ratio (PER): Weight gain (g)/Protein consumed (g)

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

2.6 Proximate composition analytical procedures

Proximate composition as moisture, ash, crude protein and lipid of fish muscles was determined by conventional method of AOAC [4] with minor modification. For determination of moisture content 10 g fish samples were taken into a hot air oven at a temperature of 105°C for 24 h. until constant weight was obtained. A total of 5 g samples were taken into a muffle furnace at a temperature of 600°C for 6 h. for ash content measurement. Kjeldahl method was used to determine crude protein content of fish samples. For most routine purposes, the percent of protein in the sample was then reckoned by multiplying the percent of nitrogen with protein conversion factor of 6.25 for fish. A ground joint soxhlet apparatus was used for the determination of lipid content.

2.7 Statistical analyses

Statistical analysis were performed using one way analysis of variance (ANOVA) and Duncan's multiple Range Test was used to determine differences between treatments means at significance rate of $p<0.05$. All statistics were carried out using Statistical Package for Social Science (SPSS) version 23.

3. Results and Discussion

3.1 Growth and feed utilization

Highest final weight of Pabda was found in T_2 (5.81±0.11 g) than those of T_1 (4.70±0.49 g), T_4 (5.24±0.03 g) and T_3 (5.29±0.15 g) (Table 2). Rahman *et al.* (2018) [18] who reported that the final weight of *Ompok pabda* fed with four different levels of probiotic 0g (PRO_0), 0.6g (PRO_1), 0.8g (PRO_2) and 1.0g (PRO_3) probiotic/kg feed for 60 days 32.49±0.60 g, 37.69±1.13 g, 41.27±2.30 g, and 34.28±1.93 g respectively in treatments PRO_0, PRO_1, PRO_2. Ali *et al.* (2018) [3] reported that final weight of *Mystus cavasius* fry fed with selected probiotics for 60 days C (control) 3.94±0.19 g, T_1 (Bactocell) 5.05±0.18 g, T_2 (Bacillus) 4.72±0.22 g, T_3 (Levucell) 4.90±0.14 g and T_4 (Mixture) 4.80±0.20 g.

Weight gain % of Pabda in different treatments was 257.90±36.84, 343.40±5.47, 303.01±12.36 and 299.62±4.11 in T_1, T_2, T_3 and T_4 respectively. Which is in aggrement with the findings of Ali *et al*. (2018) [3] who reported that weight gain % of *Mystus cavasius* fry fed with selected probiotics for 60 days C (control) 271.69±0.09, T_1 (Bactocell) 380.94±0.18, T_2 (Bacillus) 345.29±0.5, T_3 (Levucell) 366.67±0.04 and T_4 (Mixture) 348.59±0.12. Rahman *et al.* (2018) [18] reported that probiotic feed PRO_2 (0.8g) fed *O. pabda* fish showed the higher weight gain 16.76±2.76 g followed by PRO_1 (0.6g) 12.32±1.0 g, PRO_3 (1.0 g) 10.95±2.53 g.

The Specific growth rate (SGR) was ranged from 1.41-1.65. The Specific growth rate (SGR) of Pabda in different treatments was 1.41±0.12, 1.65±0.02, 1.55±0.04 and 1.54±0.01 in T_1, T_2, T_3 and T_4 respectively (Table 2). Ali *et al*. (2018) [3] reported that the specific growth rate of *Mystus cavasius* fry 2.19-2.62 by using probiotics supplemented feed. Saini *et al.* (2014) [20] reported 0.32-0.47 SGR of *L. ruhita*, fed the diet with graded levels of probiotics. Rahman *et al.* (2018) [18] reported that probiotic feed PRO_2 (0.8g) fed *O. pabda* fish showed the higher SGR 0.76. The range of SGR was (0.53-0.76).

Lowest FCR was found in T_2 (1.36±0.04) and highest in T_1 (1.86±0.21). FCR in four treatments were 1.86±0.21, 1.36±0.04, 1.55±0.08 and 1.55±0.09 in T_1, T_2, T_3 and T_4, respectively (Table 2). Rahman *et al.* (2018) [18] reported that probiotic feed PRO_0 (control) fed *O. pabda* fish showed the higher FCR 2.34 and lowest FCR was found 1.98 in PBO_3 (1.0g). Ali *et al*. (2018) [3] reported that FCR were significantly (P<0.05) lower in *Mystus cavasius* fishes of T_1 group fed with probiotics Bactocell compared with the other groups. The range of FCR was (1.59-2.01). A research done by Arslan (2004) [5] that involved *Lactobacillus bulgaicus* on carp, a low FCR was observed among the probiotics treated fish. These findings are consistent with the findings of the present study.

The protein efficiency ratio (PER) of Pabda was found 1.52±0.16, 2.10±0.07, 1.81±0.09 and 1.83±0.10 in T_1, T_2, T_3 and T_4 respectively. The PER was higher in T_2 (2.10) and lower in T_1 (1.52) (Table 2) which was coincided with the findings of Ali *et al.* (2018) [3] who recorded PER (1.98-2.50) by using probiotics supplemented feed in *Mystus cavasius* fry. Saha and Khatun (2014) [19] recorded PER of 2.81-3.97 in earthen pond at $5/m^2$ density for a period of 105 days supplying floating feed supplemented with probiotics in *O. niloticus*.

Higher survival of Pabda was found in T_2 (94.44 %) and T_3 (94.44 %) and lower in T_1 (92.59 %) and T_4 (92.59 %). This is an agreement with the findings of Munirasu *et al.* (2017) [14] who reported 96-99% survivality of *L. rohita* by using probiotics mixed feed. Ahmed *et al.* (2014) [1] reported that the overall survival of Tilapia was 95.76-97.54% by using commercial floated feed with probiotics in cages at Dakatia river.

3.2 Proximate composition

Moisture content is expressed as the amount of water as a percentage (%) and the remaining portion is dry matter content. The major component of fish muscle is moisture. In the present study the amount of moisture content in Pabda (*Ompok pabda*) was varied from 74.38±0.10 to 75.83±0.12 % (Table 3). The moisture content was obtained 74.84±0.17, 74.38±0.10, 74.46±0.11 and 75.83±0.12 % in T_1, T_2, T_3 and T_4, respectively. Nwanna *et al*. (2017) [15] found (70.03-73.71 %) moisture content by using different probiotics level in African catfish *Clarias gariepinus* Juveniles. Ali *et al*. (2018) [3] reported (75.04-76.56 %) moisture content of *Mystus cavasius* fry, fed with selected probiotics and control group for 60 days. These findings are consistent with the findings of the present study. Pal *et al.* (2017) [16] observed 74.99 % and 75.58% moisture content in farm and wild Pabda.

Final ash content of Pabda was ranged from 3.33±0.46 to 4.25±0.14 %. The ash content was obtained 4.25±0.14, 3.84±0.03, 3.33±0.46 and 3.58±0.13 % in T_1, T_2, T_3 and T_4, respectively (Table 3). Ali *et al*. (2018) [3] reported (3.47-3.60 %) ash content was found in *Mystus cavasius* fry, fed with selected probiotics and control group for 60 days. Nwanna *et al*. (2017) [15] found (4.43-5.66 %) ash content by using different probiotics level in African catfish *Clarias gariepinus* juveniles. These findings are consistent with the findings of the present study. Pal *et al.* (2017) [16] observed 1.07 % and 1.06 % ash content in farm and wild Pabda (*Ompok bimaculatus*).

Mean protein content of Pabda were 14.97±0.08, 15.69±0.10, 16.04±0.13 and 15.05±0.09 % in T_1, T_2, T_3 and T_4, respectively (Table 3) which was similar with the findings of Ali *et al*. (2018) [3] who reported (14.89-15.68 %) protein content was found in *Mystus cavasius* fry, fed with selected probiotics and control group for 60 days. Saini *et al.* (2014) [20] recorded 20.12-23.98 % protein between probiotic supplemented diet and control group of *L. rohita*. Pal *et al.* (2017) [16] observed 16.38% and 16.77 % protein content in farm and wild Pabda (*Ompok bimaculatus*).

Average lipid value of Pabda in T_1, T_2, T_3 and T_4 were, 4.87±0.09, 5.08±0.14, 5.82±0.14 and 5.38±0.14 %, respectively (Table 3). Saini *et al*. (2014) [20] reported that lipid content in *L. rohita* fed probiotics supplemented diet and only supplementary diet was 6.30-6.87 %, which is higher than the present findings. Pal *et al.* (2017) [16] observed 7.06% and 6.36% lipid content in farm and wild Pabda (*Ompok bimaculatus*). Ali *et al*. (2018) [3] who reported (3.20-3.95 %) lipid content was found in *Mystus cavasius* fry, fed with selected probiotics and control group for 60 days.

In the present study, supplementation of 0.2% dietary probiotics is considered as the best level for juvenile Pabda. Rahman *et al.* (2018) [18] also found 0.8 g/kg dietary probiotics is optimum for better reproductive performances of Pabda. African catfish (*Clarias gariepinus*) showed maximum performances with the inclusion level of probiotics (*Lactobacillus plantarum)* at 10^3 cfu/g in their diets Nwanna *et al*. (2017) [15]. Dawood *et al*. (2016) [9] concluded that incorporation of 10^4 to 10^6 cells/g LR (*Lactobacillus rhamnosus)* would be a more suitable supplement to enhance growth of Red Sea Bream. Slight variation of the optimum

probiotics supplementation level in the current study compared to the aforementioned studies might be due to the variation of the cultured species, age and the types of probiotics used.

Table 2: Growth and feed utilization of Pabda (*Ompok pabda*) in different treatments.

Parameters	T_1 (PB0)	T_2 (PB0.2)	T_3 (PB0.4)	T_4 (PB0.8)
Mean initial weight (g)	$1.31^a \pm 0.00$	$1.31^a \pm 0.01$	$1.31^a \pm 0.01$	$1.31^a \pm 0.01$
Mean final weight (g)	$4.70^c \pm 0.49$	$5.81^a \pm 0.11$	$5.29^a \pm 0.15$	$5.24^b \pm 0.03$
Weight gain %	$257.90^c \pm 36.84$	$343.40^a \pm 5.47$	$303.01^b \pm 12.36$	$299.62^b \pm 4.11$
SGR (% /day)	$1.41^b \pm 0.12$	$1.65^a \pm 0.02$	$1.55^a \pm 0.04$	$1.54^a \pm 0.01$
FCR	$1.86^a \pm 0.21$	$1.36^b \pm 0.04$	$1.55^b \pm 0.08$	$1.55^b \pm 0.09$
PER	$1.52^c \pm 0.16$	$2.10^a \pm 0.07$	$1.81^b \pm 0.09$	$1.83^b \pm 0.10$
Survival (%)	92.59 ±8.50	94.44 ±5.55	94.44 ±5.55	92.59±6.41

Values in a row having same superscripts are not significantly different (P>0.05).
Values are mean ± standard deviation of three replicates.

Table 3: Proximate composition of *Ompok pabda* fry fed with different levels of probiotics (% w/w basis)**

Parameters	Initial (%)	Final (%)			
		T_1 (PB0)	T_2 (PB0.2)	T_3 (PB0.4)	T_4 PB0.8
Moisture	82.14	74.84 ± 0.17^b	74.38 ± 0.10^a	$74.46 \pm .11^a$	$75.83 \pm .12^c$
Ash	2.18	4.25 ± 0.14^c	3.84 ± 0.03^{bc}	3.33 ± 0.46^a	3.58 ± 0.13^a
Protein	12.17	14.97 ± 0.08^a	15.69 ± 0.10^b	16.04 ± 0.13^c	15.05 ± 0.09^a
Lipid	3.62	4.87 ± 0.09^a	5.08 ± 0.14^a	5.82 ± 0.14^c	5.38 ± 0.14^b

Values in the same row having same superscripts are not significantly different (P>0.05).
** Values are mean ± standard deviation.
The initial samples were not included in statistical analysis.

4. Conclusions

Supplementation of probiotics with feed play a significant role in increasing the growth, feed utilization and the whole fish body composition of Pabda. In respect of Pabda weight, SGR (%/day), FCR, PER and survival rate (%), T_2 treatment performed the best growth performance. But diets with probiotics were not significantly different (P>0.05) in specific growth rate, feed conversion ratio. Amount of ash is higher in T_1 (0% probiotics). Moisture is high in T_4 (0.8% probiotics), protein content is high in T_3 (0.4% probiotics) and lipid content is high in T_3 (0.4% probiotics). Fish fed diets with probiotics had better growth, feed utilization and survivality when compared with those fed diets without probiotics. On the basis of growth performance 0.2% of probiotics (T_2) is optimum for incorporation in diets of *Ompok pabda*. An increase level of probiotics supplemented in diets may not promote the growth and proximate composition of *Ompok pabda* in this study.

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