Investigation on the growth performances of selected probiotic supplemented tilapia (*Oreochromis niloticus*) and walking catfish (*Clarias batrachus*) fingerlings

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**ABSTRACT**

The importance of probiotics in nutrition and health of aquaculture species is widely recognized. The current study was conducted to evaluate the effects of two commercial probiotic supplemented diets on the growth performance of monosex tilapia, *Oreochromis niloticus* and walking catfish, *Clarias batrachus* fingerlings. A 4-week long feeding trial was conducted with eight 35 L capacity rectangular glass aquaria using 48 *O. niloticus* (13.34 ± 2.19 g) and 48 *C. batrachus* (13.26 ± 1.50 g). Two available commercial probiotics were added with commercial fish feed (starter 1) and introduced in aquaria twice-a-day at 3% body weight in three forms; feed +1% probiotic Eskalina, Eskayef (organic spirulina 100%) (D1), feed + 1% mixed bacterial probiotic BioFav Aqua, Novertis (D2), feed + 1:1 ratio mixture of probiotics 0.5% Eskalina + 0.5% Bio BioFav Aqua (D3) and feed without probiotic (D4) as control. Proximate composition of feed samples was examined. Morphometric measurements of experimental fish and water quality parameters were determined weekly. The results showed that net weight gain, percent weight gain, specific growth rate (SGR) and protein efficiency ratio (PER) were found higher in *O. niloticus* and *C. batrachus* fed with diet D3 and diet D1, respectively. On the other hand, control diet, D4 showed lower growth and feed efficiencies for both fishes compared to other diets. The percentage of survival was independent of treatments. Whole body protein, fat and ash contents were higher and moisture content was lower in both fish fed with diet D1 followed by D3, D2 and control. These results suggest that diets incorporated with probiotics may be used as regular feed in *O. niloticus* and *C. batrachus* culture for better feed efficiency and growth performance. Spirulina supplemented diet, D1 and mixture of spirulina + probiotic bacteria supplemented diet, D3 appear to be more adequate respectively for the commercial farming of *C. batrachus* and *O. niloticus*.

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**INTRODUCTION**

Aquaculture is one of the fast-growing systems in the world, which has emerged as an industry to supply protein rich food throughout the world (Prasad 1996). Fish is recognized as an important dietary animal protein source in human nutrition. Production of aquatic species through freshwater fisheries and aquaculture for protein supply is being encouraged throughout the world. This activity requires high-quality feeds with a high protein content, which should contain not only the necessary nutrients but also complementary additives to keep organisms healthy and promote favourable growth. Functional additive, like probiotics is a new concept in aquaculture (Li and MGatlin III 2004) where the additions of microorganisms on diets showed a positive effect on growth caused by the best use of carbohydrates, proteins and energy.

According to the World Health Organization (FAO/WHO 2001) probiotic bacteria are defined as ‘live microorganisms which when administered in adequate amounts confer a health benefit on the host’. Microbial products, such as probiotics, are considered valid alternatives to the prophylactic use of
chemicals in aquaculture practices (Gatesoupe 2008; Merrifield et al. 2010). Recent studies have clearly demonstrated the beneficial effects of these feed additives on immune system modulation, stress tolerance and growth rate of farmed fishes (Carnevali et al. 2004; Carnevali et al. 2006; Picchietti et al. 2007; Dimitroglou et al. 2011). The use of probiotic in feeds to improve growth of different fish species including African catfish (Al-Dohail et al. 2009), Senegalese sole (Sáenz de Rodríguez et al. 2009), Nile tilapia (Lara-Flores et al. 2003; 2010; El-Haroun et al. 2006), Japanese flounder (Taoka et al. 2006), gilthead sea bream and sea bass (Carnevali et al. 2006) has been investigated. Also there has been an increasing interest in the possible use of probiotics in south-east Asian aquaculture, including application in freshwater teleosts such as Indian major carps, *Catla catla*, *Labeo rohita* and *Cirrhinus cirrhosus*, the Chinese carps, *Hypophthalmichys molitrix* and *Ctenopharyngodon idella*, common carp, *Cyprinus carpio*, tilapia, *Oreochromis mossambicus*, Nile tilapia *O. niloticus*, walking catfish, *Clarias batrachus*, the murrel, *Channa punctatus*, and rainbow trout, *Oncorhynchus mykiss* (Bairagi et al. 2002; Panigrahi et al. 2005; El-Haroun et al. 2006). The effects of probiotics have been linked to modulation of gut microbiota and establishment of the beneficial microorganisms, higher specific and total digestive enzyme activities, in the brush border membrane, which increases the nutrient digestibility and feed utilization (Verschueren et al. 2000; Balc’azar et al. 2006; Kesarcodici-Watson et al. 2008).

In the aquaculture of Bangladesh, monosex tilapia (*O. niloticus*), locally known as tilapia and walking catfish (*C. batrachus*), locally known as magur, are two most preferred farmed fish species, because of their high production in mono and polyculture operation and have greater acceptability to the consumers for delicious test. Although the culture of tilapia and magur has already been flourishing in Bangladesh, farmers are still facing problem due to high cost and inadequate supply of quality fish feeds and feed ingredients. The existing situation is now demanding to develop a good quality hazard free diet treated with probiotics to introduce probiotics cells to the fish gut in order to establish a balanced gastrointestinal microbial flora for the improvement of the digestive function or immune system responses in tilapia and magur. Considering all, the present study has been designed to prepare suitable feed supplemented with available commercial probiotics and to evaluate the feed efficiency and growth performances of monosex tilapia and walking catfish fingerlings fed with formulated feed.

**MATERIALS AND METHODS**

**Study Area and Duration**

The experiment was conducted at the in-vivo aquarium trial room of the Fish Disease Laboratory and Fish Nutrition Laboratory, Faculty of Fisheries, Bangladesh Agricultural University (BAU), Mymensingh 2202 for a period of 4 weeks from 22 February 2016 to 21 March 2016.

**Fingerling Collection and Acclimatization**

Healthy fishes (*O. niloticus* having average weight 13.34 ± 2.19 g and *C. batrachus* having average weight 13.26 ± 1.5 g) were obtained from private fish hatcheries adjacent to the BAU, Mymensingh 2202 campus and transported to the laboratory in oxygenated plastic bags. They were allowed to acclimatize in the laboratory conditions for a week with continuous oxygen supply and fed commercial pelleted feed (floating feed; 3% of fish body weight) twice daily at 9:30 am and 5:00 pm prior to use for the experiment.

**Experimental Setup**

**Stocking of fish fingerling**

Experiments were conducted in eight glass aquaria (35 L capacity) filled with 30 L fresh and clean ground water. Forty eight *O. niloticus* and forty eight *C. batrachus* fingerlings were divided into four equal groups, respectively so that each aquarium contained 12 fishes. Stocking density was 1 fish per 2.5 L, and all aquaria were provided with continuous aeration. The fish were fed with commercial diet (floating feed) supplemented with commercial probiotics. Control groups of fish received same commercial diet without probiotics. Each aquarium was cleaned daily by 75% water exchange after siphoning out fish feces and uneaten feed. Water temperature was measured daily.

**Preparation of probiotic supplemented feed**

Two commercially available probiotic powder commonly used as food additives viz., Eskalina (organic spirulina 100%, Eskayef) and BioFav Aqua (mixture of Bacillus and other probiotic bacteria, Novertis) (Fig. 1), were used in this experiment. Water from the boiling rice was taken, cooled to room temperature and 1% of each probiotic powder was mixed well separately with it to make the probiotic suspension. Then, 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. The base diet was mixed with 1% Eskalina (D1), 1% BioFav Aqua (D2) and with a 1:1 mixture of both 0.5% Eskalina and 0.5% BioFav Aqua in equal amounts (D3). The untreated basal diet was served as control (D4) (Fig. 2). No replication was used during the feeding experiment.

**Fig. 1 Commercial probiotics used in the present study. a) Eskalina (organic spirulina 100%, Eskayef), b) BioFav Aqua (mixture of Bacillus and other probiotic bacteria, Novertis)***

**Fig. 2 Commercial feed mixed with probiotics used in the present study. a) diet 1 (D1): starter 1 + 1% Eskalina, b) diet 2 (D2): starter 1 + 1% BioFav Aqua, c) diet 3 (D3): starter 1 + 0.5% Eskalina + 0.5% BioFav Aqua, d) diet 4 (D4): starter 1 without probiotic (control diet)**
Feeding experiment

Fish in the aquarium were fed with experimental diets twice daily in the morning at 9:30 am and afternoon at 5 pm at a rate of 3% of their body weight and checked regularly whether the feed was consumed or not.

Sampling of Fish and Water

Fish were sampled once a week, caught by the hand held scoop net and body weight of the individual fish was measured carefully using an electric balance. Water quality parameters of both the system e.g., dissolved oxygen (DO) (mg/L), water temperature (°C) and pH were monitored weekly throughout the study period. The dissolved oxygen values were recorded using a portable DO meter and denoted as milligram per liter (mg/L). Water temperature was measured by hand thermometer and denoted as °C. Water pH of individual aquarium was recorded using a portable pH meter (Hanna).

Analytical Methods

Proximate composition of feed samples

The probiotics supplemented diets were analyzed for protein, lipid, carbohydrate, ash, moisture and crude fibre content. Analysis of the proximate composition of feed samples was done according to AOAC (1990) in the Fish Nutrition Laboratory, Department of Aquaculture, BAU, Mymensingh.

Estimation of protein

Crude protein of the samples was estimated by using Kjeltc Auto 1030 Analyzer. Calculations of crude protein of the samples were done using the following formula:

\[
\% \text{ Nitrogen} = \frac{0.014 \times N \times (T-B)}{\text{Weight of sample}}
\]

\[
\% \text{ Crude protein} = \% \text{ Nitrogen} \times 6.25 \text{ (for animal)} = \text{Nitrogen} \times 5.58 \text{ (for plant)}
\]

Where,

- \( T \) = Reading of titrate of samples
- \( B \) = Reading of titrate of blank samples
- \( N \) = Normality of HCl and
- 0.014 = Millieqivalent wt. of nitrogen (g)

Estimation of lipid

To determine crude lipid, the Soxhlet apparatus was used for solvent extraction of lipid. Calculation of lipid was done by using the following formula:

\[
\% \text{ Crude lipids} = \frac{\text{Weight of breaker with lipid after oven dry} - \text{Initial wt of breaker}}{\text{Weight of sample}} \times 100
\]

Estimation of carbohydrate (CHO)

Carbohydrate content of the samples was determined as total carbohydrate by difference, that is, subtracting the measured protein, fat, ash, and moisture from 100 (Pearson 1970).

Estimation of moisture, ash and crude fibre content

Moisture, ash and crude fibre contents of experimental feed were determined using AOAC (1990) method. The percentage of moisture, ash and crude fibre contents were calculated by using respective formulae.

\[
\% \text{ Moisture content} = \frac{X-Y}{X} \times 100
\]

Where,

- \( X \) = Weight of sample (g) before drying and
- \( Y \) = Weight of samples (g) after drying

\[
\% \text{ Ash content} = \frac{W_1 - W_2}{W_0} \times 100
\]

Where, \( W_0 \) = Weight of sample

- \( W_1 \) = Weight of crucible with ash
- \( W_2 \) = Weight of empty crucible

\[
\% \text{ Crude fibre content} = \frac{\text{Loss in weight noted}}{\text{Weight of samples taken}} \times 100
\]

Morphometric measurements of the fingerlings

Every week, the fishes were measured for wet body weight. After obtaining the data, wet weight gain was calculated using following formula.

\[
\text{Wet weight gain (g)} = \text{Final weight (g)} - \text{Initial weight (g)}
\]

Percentage (%) weight gain = \[
\frac{\text{Mean final weight (g) - mean initial weight (g)}}{\text{Mean initial weight (g)}} \times 100
\]

Growth Parameters and Rate of Feed Intake

The fishes in each treatment were counted and weighed on termination of the experiment. Growth performance and feed efficiency were determined by evaluating a number of growth and nutrient utilization indices, including net weight gain, percentage weight gain, specific growth rate (SGR), feed conversion ratio (FCR), feed conversion efficiency (FCE), protein efficiency ratio (PER) and energy retention. The growth parameters and feed utilization were calculated as follows:

\[
\text{SGR} = 100 \left( \frac{\text{ln} W_2 - \text{ln} W_1}{T} \right) T^{-1}
\]

Where, \( W_1 \) and \( W_2 \) are the initial and final weights and \( T \) is the number of days of feeding.

\[
\text{FCR} = \frac{\text{Total dry feed consumption (g)}}{\text{Live weight gain (g)}}
\]

\[
\text{FCE} = \frac{\text{Live weight gain (g)}}{\text{Dry feed consumed (g)}}
\]

\[
\text{PER} = \frac{\text{Live wet weight gain (g)}}{\text{Crude protein intake (g)}}
\]

Proximate Composition of Whole Fish Samples

On the conclusion of the trials, the final body weight attained by the fish, were recorded separately for each treatment. Prior to start and at the end of the experimental period, proximate composition of fish body was determined using three fish per aquarium in terms of moisture, crude protein, crude lipid and crude ash contents (AOAC 1990).

Data Processing and Analysis

Fish weight gain, growth parameters, production and water quality was determined and expressed as mean ± (standard deviation). Data analyses performed using Microsoft Excel 2010, with an alpha set at 0.05 and 0.01 (significance at p<0.05 and p<0.01). Mean values of fish whole body proximate compositions were tested by two-way ANOVA. If there were significant differences at significant level of 0.05 and 0.01 then Duncan Multiple Range Test (DMRT) was used to compare the means to show significant differences between the treatments.

RESULTS

Water Quality Parameters

Water quality parameters play an important role in the growth and development of aquatic organisms. All the water quality parameters were within the productive range. The values of the water quality parameters of different tilapia and magur rearing aquaria are shown in Table 1. The values of dissolved oxygen varied from 4.40 to 5.60 mg/L. The highest dissolved oxygen value was 5.60 mg/l on February 29 and the lowest value was 4.40 mg/L on March 21. Water temperature ranged from 25.2°C to 31.6°C during the study period. The maximum temperature was 31.6°C on March 21, 2016 while the minimum was 25.2°C
The water pH ranged from 6.6 to 7.8 during the study period. The highest pH value was 7.8 on March 14, 2016 while the lowest pH value was 6.6 on March 21, 2016.

Table 1. Mean (± SD) of water quality parameters in different tilapia and magur rearing aquaria during the study period from February 22 to March 21, 2016

<table>
<thead>
<tr>
<th>Parameters</th>
<th>29.02.16 Mean ± SD</th>
<th>20.03.16 Mean ± SD</th>
<th>21.03.16 Mean ± SD</th>
<th>22.03.16 Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dissolved oxygen (mg/L)</td>
<td>5.02 ± 0.17</td>
<td>4.74 ± 0.33</td>
<td>4.90 ± 0.39</td>
<td>4.72 ± 0.38</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>7.3 ± 0.13</td>
<td>7.2 ± 0.18</td>
<td>7.4 ± 0.17</td>
<td>6.8 ± 0.29</td>
</tr>
<tr>
<td>pH</td>
<td>7.9 ± 0.28</td>
<td>29.9 ± 0.31</td>
<td>30.5 ± 0.39</td>
<td>31.2 ± 0.31</td>
</tr>
</tbody>
</table>

Proximate Composition of Feed Samples

Commercial pelleted fish feed mixed with probiotic Eskalina (D1) contained 30.58% crude protein, 7.20% crude lipid, 36.33% carbohydrate, 11.04% ash, 9.57% moisture and 5.20% crude fibre. The proximate composition of 7.95% crude lipid, 35.28% carbohydrate, 11.17% ash, 9.75% moisture and 4.90% crude fibre. The proximate composition of other diets. However, the growth, feed efficiency, PER were higher in magur fed with the diet D3 but moderate in magur. On the other hand, similar parameters were higher in magur fed with the diet D1 than that of other diets. However, the growth, feed efficiency, PER were lower but FCR was found higher in control diet (D4) than that of D1, D2 or D3 in both the cases of tilapia and magur. The weekly weight increments of other diets. However, the growth, feed efficiency, PER were lower but FCR was found higher in control diet (D4) than that of D1, D2 or D3 in both the cases of tilapia and magur. The weekly weight increments of other diets. However, the growth, feed efficiency, PER were lower but FCR was found higher in control diet (D4) than that of D1, D2 or D3 in both the cases of tilapia and magur. The weekly weight increments of other diets. However, the growth, feed efficiency, PER were lower but FCR was found higher in control diet (D4) than that of D1, D2 or D3 in both the cases of tilapia and magur. The weekly weight increments of other diets. However, the growth, feed efficiency, PER were lower but FCR was found higher in control diet (D4) than that of D1, D2 or D3 in both the cases of tilapia and magur. The weekly weight increments of other diets. However, the growth, feed efficiency, PER were lower but FCR was found higher in control diet (D4) than that of D1, D2 or D3 in both the cases of tilapia and magur.

Growth Parameters and Rate of Feed Intake

Growth performance, feed efficiency and survival data for fish fed with diets D1, D2, D3 and control are shown in Table 3 and Table 4. Mortality rate was found the least in diet D1 in both the cases of tilapia and magur. Net weight gain, percentage weight gain, SGR and PER was higher in tilapia fed with the diet D3 based feed for 4 weeks. However, the growth, feed efficiency, PER were lower but FCR was found higher in control diet (D4) than that of D1, D2 or D3 in both the cases of tilapia and magur. The weekly weight increments of O. niloticus in under different treatments are graphically shown in Figure 3 and Figure 4, respectively.

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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>D1</th>
<th>D2</th>
<th>D3</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude Protein</td>
<td>30.58</td>
<td>29.96</td>
<td>30.95</td>
<td>29.25</td>
</tr>
<tr>
<td>Crude lipid</td>
<td>7.20</td>
<td>7.70</td>
<td>7.95</td>
<td>6.60</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>36.33</td>
<td>36.76</td>
<td>35.28</td>
<td>36.31</td>
</tr>
<tr>
<td>Ash</td>
<td>11.12</td>
<td>11.14</td>
<td>11.17</td>
<td>11.21</td>
</tr>
<tr>
<td>Moisture</td>
<td>9.57</td>
<td>9.84</td>
<td>9.75</td>
<td>11.03</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>5.20</td>
<td>4.60</td>
<td>4.90</td>
<td>5.60</td>
</tr>
</tbody>
</table>

Table 3. Growth responses of O. niloticus fed selected probiotic based feed for 4 weeks

<table>
<thead>
<tr>
<th>Parameters</th>
<th>D1</th>
<th>D2</th>
<th>D3</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body wt. (g)</td>
<td>16.69 ± 0.86</td>
<td>12.38 ± 1.26</td>
<td>12.37 ± 0.79</td>
<td>11.91 ± 1.06</td>
</tr>
<tr>
<td>Final body wt. (g)</td>
<td>30.52 ± 0.72</td>
<td>24.06 ± 0.95</td>
<td>26.02 ± 2.49</td>
<td>20.97 ± 1.71</td>
</tr>
<tr>
<td>Net weight gain (g)</td>
<td>13.83</td>
<td>11.68</td>
<td>13.65</td>
<td>9.06</td>
</tr>
<tr>
<td>Weight gain (%)</td>
<td>82.86</td>
<td>94.35</td>
<td>110.35</td>
<td>76.07</td>
</tr>
<tr>
<td>Specific growth rate (SGR) (%) day</td>
<td>2.16</td>
<td>2.37</td>
<td>2.66</td>
<td>2.02</td>
</tr>
<tr>
<td>Food conversion ratio (FCR)</td>
<td>1.56</td>
<td>1.41</td>
<td>1.2</td>
<td>1.7</td>
</tr>
<tr>
<td>Food conversion efficiency (FCE)</td>
<td>0.64</td>
<td>0.70</td>
<td>0.83</td>
<td>0.59</td>
</tr>
<tr>
<td>Protein efficiency ratio (PER)</td>
<td>1.72</td>
<td>1.25</td>
<td>2.14</td>
<td>0.73</td>
</tr>
<tr>
<td>Survival (%)</td>
<td>91.67</td>
<td>83.33</td>
<td>91.67</td>
<td>75.00</td>
</tr>
</tbody>
</table>

Table 4. Growth responses of C. batrachus fed selected probiotic based feed for 4 weeks

<table>
<thead>
<tr>
<th>Parameters</th>
<th>D1</th>
<th>D2</th>
<th>D3</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body wt. (g)</td>
<td>14.14 ± 0.64</td>
<td>12.41 ± 0.85</td>
<td>14.82 ± 0.80</td>
<td>11.67 ± 0.89</td>
</tr>
<tr>
<td>Final body wt. (g)</td>
<td>26.68 ± 1.32</td>
<td>23.00 ± 0.79</td>
<td>26.88 ± 1.37</td>
<td>20.21 ± 1.72</td>
</tr>
<tr>
<td>Net weight gain (g)</td>
<td>12.54</td>
<td>10.59</td>
<td>12.06</td>
<td>8.54</td>
</tr>
<tr>
<td>Weight gain (%)</td>
<td>88.86</td>
<td>85.33</td>
<td>81.38</td>
<td>73.18</td>
</tr>
<tr>
<td>Specific growth rate (SGR) (%) day</td>
<td>2.27</td>
<td>2.20</td>
<td>2.13</td>
<td>1.96</td>
</tr>
<tr>
<td>Food conversion ratio (FCR)</td>
<td>1.23</td>
<td>1.53</td>
<td>1.3</td>
<td>1.75</td>
</tr>
<tr>
<td>Food conversion efficiency (FCE)</td>
<td>0.81</td>
<td>0.65</td>
<td>0.77</td>
<td>0.57</td>
</tr>
<tr>
<td>Protein efficiency ratio (PER)</td>
<td>2.57</td>
<td>3.09</td>
<td>2.57</td>
<td>1.74</td>
</tr>
<tr>
<td>Survival (%)</td>
<td>91.67</td>
<td>91.67</td>
<td>83.33</td>
<td>66.67</td>
</tr>
</tbody>
</table>

Figure 3. The mean weekly response by O. niloticus on the experimental diets for 4 weeks

Figure 4. The mean weekly response by C. batrachus on the experimental diets for 4 weeks
Effects of Different Probiotic Based Diets on Fish Body Compositions

The effect of different diets on the body composition of tilapia and magur are presented in Table 5 and Table 6, respectively. The carcass protein content in fish body were ranged between 14.99 to 15.52% in tilapia and 13.32 to 14.30% in magur. The body composition showed that protein deposition was increased but lipid and moisture contents were found deceased both in tilapia and magur fed with different diets. No significant difference was found for protein contents in fish fed with different diets. Also, no significant variations was found among the lipid contents of fishes fed with the diets. However, significant lower moisture content of magur was found when fed with D1, D2 and D3 although tilapia did not show significant variation in moisture contents fed with the same diets. Body ash contents were not affected by the treatments in respect of tilapia and magur.

Table 5. Proximate composition (% wet wt. basis) of O. niloticus at the beginning and end of the experiment

<table>
<thead>
<tr>
<th></th>
<th>Initial (all fish)</th>
<th>D1</th>
<th>D2</th>
<th>D3</th>
<th>Control</th>
<th>Level of sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>77.85</td>
<td>73.82±0.92</td>
<td>74.00±0.85</td>
<td>74.60±0.72</td>
<td>74.6±0.67</td>
<td>NS</td>
</tr>
<tr>
<td>Crude Protein</td>
<td>13.10</td>
<td>13.52±0.90</td>
<td>14.99±0.95</td>
<td>15.01±0.96</td>
<td>15.24±0.75</td>
<td>NS</td>
</tr>
<tr>
<td>Crude lipid</td>
<td>4.84</td>
<td>3.81±0.82</td>
<td>3.58±0.71</td>
<td>3.76±0.81</td>
<td>3.10±0.98</td>
<td>NS</td>
</tr>
<tr>
<td>Ash</td>
<td>4.21</td>
<td>4.85±0.72</td>
<td>4.43±0.94</td>
<td>4.63±0.66</td>
<td>5.01±0.61</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data present the mean S. E. of three replicates. Values on the same row with same subscript do not differ significantly whereas Values with different subscripts are significantly different (as per DMRT).

* = significant at 5% level of probability

Table 6. Proximate composition (% wet wt basis) of C. batrachus at the beginning and end of the experiment

<table>
<thead>
<tr>
<th></th>
<th>Initial (all fish)</th>
<th>D1</th>
<th>D2</th>
<th>D3</th>
<th>Control</th>
<th>Level of sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>78.34</td>
<td>73.34±0.85</td>
<td>75.03±1.34</td>
<td>73.65±0.82</td>
<td>76.01±0.94</td>
<td>*</td>
</tr>
<tr>
<td>Crude Protein</td>
<td>11.93</td>
<td>14.3±1.14</td>
<td>13.58±0.79</td>
<td>13.86±0.72</td>
<td>13.32±0.61</td>
<td>NS</td>
</tr>
<tr>
<td>Crude lipid</td>
<td>7.02</td>
<td>5.91±0.98</td>
<td>5.06±1.07</td>
<td>5.59±1.08</td>
<td>5.08±1.07</td>
<td>NS</td>
</tr>
<tr>
<td>Ash</td>
<td>2.71</td>
<td>3.47±1.12</td>
<td>3.32±0.77</td>
<td>2.90±0.72</td>
<td>3.59±0.67</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data present the mean S. E. of three replicates. Values on the same row with same subscript do not differ significantly whereas Values with different subscripts are significantly different (as per DMRT).

* = significant at 5% level of probability

DISCUSSION

Aquaculture sector has developed strategies in various countries to improve fish health and fish growth. Among the strategies, the more promising one is the use of probiotics. Many studies on probiotics in aquaculture have measured the survival of probiotic organisms in fish gut (Andlid et al. 1998), or evaluated the beneficial effect of probiotic on health management, disease resistance and immune response of fishes (Li and Gatlin III 2004; Shelby et al. 2006). Other important effect of probiotic use, that it is not extensively studied, but demonstrated an important effect, is the feed efficiency and the growth promotion (Gatesoupe 2002; Lara-Flores et al. 2003, 2010). In this investigation the commercial probiotics treated fish diets (D1, D2 and D3) as well as commercial fish feed without probiotics (D4) were analyzed for their potential growth promoting effects on tilapia and magur fingerlings. According the growth effects of commercial probiotic supplemented feed, higher levels of growth assessing parameters were found for the fishes fed with experimental diets as compared to the control that clearly demonstrated the potentiality of the reported probiotics.

Water quality parameters are one of the most important factors for successful aquaculture. The optimum level of dissolved oxygen (DO) is required for fish culture. In the present study, DO concentration in water varied from 4.40 to 5.60 mg/L (Haque et al. 2000) and Ali et al. (2008) found more or less similar results. Water temperature is one of the most important factors, which influence the growth, reproduction and other biological activities of fish. The temperature varied from 25.2°C to 31.6°C during the present study. Faruk et al. (2012) reported that in Bangladesh, growth of tilapia can be achieved between the temperature range of 25-29°C. Moreover, temperature reported by Collins (1973) for the culture of C. batrachus was also found similar to the present study. pH indicates acidity-alkalinity condition of a water body. It is called the productivity index of water body. Most water bodies have pH within the ranges of 6.5 to 8.5. The slightly alkaline pH is most suitable for fish culture. By contrast, acidic pH of water reduces the growth and metabolic rate and other physiological activities of fishes (Swingle 1967). In present study, the range of pH varied from 6.6 to 7.8 that ultimately showed similar range reported by Bhuiyan (1970) and Wahab et al. (1995).

In the present study, better growth performance and SGR were observed in tilapia and magur fingerlings with the probiotics supplement diet viz., D1, D2 and D3 compared with the control diet. Similar observations have been reported on African catfish, C. gariepinus (Al-Dohail et al. 2009; Ayoola et al. 2013) and L. rohita (Mohamed et al. 2007). They reported that growth performance in the fishes were significantly (P<0.05) higher in the probiotic treated groups than the control when Lactobacillus acidophilus and L. delbrueckii were used as a probiotics as feed additives in their formulated diets. This gives an indication of an improvement in the health and growth performance of fish despite the differences in the methods and species used in the present study. The improvement in growth may, however, be related to the improvement in the intestinal microfloral balance as reported by Fuller (1989).

As a single probiotic additive, spirulina exhibited very interesting results in the present study by enhancing growth in both experimental fishes. Nandeesh et al. (2001) studied the influence of Spirulina platensis meal on the growth of C. catla and L. rohita for 90-days culture period. The specific growth rate and protein efficiency ratio recorded in rohu improved with higher levels of Spirulina inclusion, while in catla they did not differ significantly from the control. However, Jana et al. (2014) observed that length and weight gain, and survival of P. sutchi were significantly the best with the addition of Spirulina content in the feed which is very much suited to the growth enhancement of C. batrachus in the present study.
The study results also showed that FCR were better in fish fed on the probiotics treated diets (D1, D2 and D3) compared to the control diet. The results are in agreement with the findings on Nile tilapia (Lara-Flores et al. 2003; Mohamed et al. 2007), African catfish (Al-Dohail et al. 2009; Ayoola et al. 2013) and common carp (Noh et al.1994; Yanbo and Zirong 2006). In the present study, higher protein utilization, determined in terms of PER, increased to some extent in fish maintained with the probiotic supplemented diets than in the fish maintained with the probiotic free control diet. This result also agree to the findings of Lara-Flores et al. (2003) where better PER was found in Nile tilapia fed diets supplemented with commercial probiotics (Streptococcus faecium) and Lactobacillus acidophilus. Similar observations have been reported on African catfish fed diets supplemented with probiotics, bacterial genus Lactobacillus Al-Dohail et al. 2009; Ayoola et al. 2013).

**CONCLUSION**

The specific functions of probiotics in aquaculture may not be denied. It could be concluded that both Spirulina based commercial probiotic and mixed bacterial probiotics were found beneficial for rearing of tilapia and magur fingerlings when administered as a feed additive. But the combination of these two probiotics produced more beneficial effects by enhancing the growth performance and survival rate of fish. More works are needed to assess the Spirulina and mixed bacterial probiotics effects on growth and immune responses in fishes, including *O. niloticus* and *C. batrachus*.

**CONFLICT OF INTEREST**

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

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