Effect of Different Probiotics on Growth, Survival and Production of Monosex Nile Tilapia (*Oreochromis niloticus*)

**MS Khatun and Dr. SB Saha**

**Abstract**

Effect of different oral probiotics on growth, survival and production of monosex Nile tilapia (*Oreochromis niloticus*) under brackishwater pond was studied in eight brackishwater ponds, where tilapia fries with the average body weight (ABW) of 0.15 g were stocked at a density of 5 Nos/m². Salinity of the ponds varied from 10 ppt to 16 ppt. Fishes were fed with floating feed containing 28% protein. Three types of probiotics viz., Safegut (T1), Zymetin (T2), Probio-aqua (T3) were supplemented @ 5 mg or ml per kg feed. Control (T4) ponds were not supplemented with probiotics. After 105 days of culture, highest production (8681±626.50 kg/ha) and survival (92.35±6.41) was found in T3, where Probio-aqua was supplemented with diet. Control (T4) produced lowest production (6967±982.17 kg/ha) but there was no significant (*P* < 0.05) difference among treatments.

**Keywords:** Production, tilapia, monosex, probiotics, brackishwater

**Introduction**

Probiotics are live microorganisms that act beneficially in the host, promoting the balance of the intestinal microbiota, favoring the health of the animals (Fuller, 1989) [20]. Nayak (2010) [38] stated that an ideal probiotic, irrespective of its source must be able to colonize and multiply in the intestine of the host. There are a wide range of microalgae (*Tetraselmis*), yeast (*Debaryomyces, Phaffia* and *Saccharomyces*) and gram positive (*Bacillus, Lactococcus, Micrococcus, Carnobacterium, Enterococcus, Lactobacillus, Streptococcus, Weissella*) and gram negative bacteria (*Aeromonas, Alteromonas, Photobacterium, Pseudomonas and Vibrio*) that have been evaluated as a probiotics (Gastesoupe, 1999) [22].

Several studies have exhibited promising results of the use of probiotics in fish, mollusk, crustacean and amphibian farming Verschuere et al., 2000 [55]; Ringo and Gastesoupe, 1998 [37]; Irianto and Austin, 2002 [28]; Dias et al., 2008 [13]; Kesarcodi-Watson et al., 2008 [29]; El-Rhman et al., 2009 [17]; Zhou et al., 2009 [62], which enables the probiotics to substitute the antibiotics as growth promoters. In fish, they are usually administered orally in order to improve the microbial flora of the intestine (Nageswar and Babu, 2006; Sahu et al., 2008) [37, 48]. Nile tilapia is an economically important cultured species in several areas of the world (El-Husseny et al., 2007; El-Saidy and Gaber, 2005) [16, 18]. They are resulting in a greater presently cultured in virtually all types of production systems, in both fresh and salt water and in tropical, subtropical and temperate climates (Fitzsimmons et al., 2006) [19]. Tilapia dominate both small and large scale aquaculture in many tropical and subtropical countries, both as low price commodity for mass consumption as a staple protein source and as a high value, upscale product for export markets (Lim and Webster, 2006) [32].

A study evaluating the least—cost dietary protein level for four species of tilapia (*Orechromis mossambicus, O. niloticus, O. aureus and Tilapia zillii*) showed that the dietary protein level from 34% to 36% provided maximum growth of young tilapia (1.5 g), but the most cost-effective protein level was 25% to 28% (De Silva et al., 1989) [12].

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The probiotics of live microbes have shown their effectiveness to mitigate the effects of stress, resulting in a greater production of Nile tilapia (Ghazalah et al, 2010) [23]. Olvera et al., (2001) [43] concluded that yeast have a positive effect on fish performance when cultured under stress condition of lowering dietary protein, leading to improving growth and feed efficiency. In contrast, Hidalgo et al., (2006) [32] found that growth and feed conversion of juvenile dentex...
(Dentex dentex) were not significantly influenced by probiotics which is in agreement with the findings, Shelby et al. (2006) [50] who found that the probiotic used with juvenile Nile tilapia diet had lack effect on specific growth promoting or immune stimulating aspects. On the other hand, many studies concluded the positive effect of using viable microorganisms in probiotic mixtures into diets of fish Li and Gatlin 2004 [31], Brunt and Austin, 2005 [10]; Pangrahi et al., 2005 [44]; Barnes et al., 2006 [6]; Abo–State et al., 2009 [1]. Mohamed A Essa et al (2010) [15] showed that all the diets containing different probiotic groups significantly (P<0.05) improved Nile tilapia growth and feed utilization compared to the control diet. This study was conducted to evaluate the effect of dietary supplementation of commercial probiotics on growth and production of Nile tilapia (Oreochromis niloticus) in brackishwater environment.

Material and Methods
The study was carried out in eight earthen ponds of the Bangladesh Fisheries Research Institute, Brackishwater Station, Paikgacha, having area of 1000 m² each. The study was done from 1st April to 15th July, 2014.

Experimental design and condition: The study was conducted according to the following experimental design (table 1).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Probiotics</th>
<th>Mode of application</th>
<th>Stocking density (No/m²)</th>
<th>Replications</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>Safeguard (Bacillus subtilis, B. licheniformis, L. sporogrwnrs, S. boulardii, S. cerevisiae, Aspergillus oryzae, A. niger, Vit. B1 &amp; B6, coated vit. C and some enzymes)</td>
<td>Dietary supplementation</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>T2</td>
<td>Zymetin (Bacillus mesentericus, Streptococcus faecalis, Clostridium butyricum)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>Probio-Aqua (Rhodopseudomonas palustris &amp; some basic media)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>No probiotics (control)</td>
<td></td>
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</tbody>
</table>

Pond preparation and fry stocking
Soil of each pond was sun-dried and treated with lime (Cao@ 250 kg/ha). A temporary in-pond nursery (25 m²) was prepared at one corner in each pond by erecting nylon net frequently following standard methods (APHA 1992) [3].

Bacterial count
Total Heterotrophic Bacteria (THB) and pathogenic bacteria mainly Vibrio sp. of both water and soil of the experimental ponds was tested using pour plate bacterial culture method. Nutrient agar media and TCBS (Thiosulfate citrate bile salt) agar media was used for culturing THB and Vibrio sp. respectively. Viable colony was counted using a colony counter.

Harvesting
After 105 days of culture, all fishes were harvested by draining out ponds and growth and production were estimated.

Data analysis
Growth performances were determined and feed utilization was calculated as following:

- Daily weight gain (DWG), Specific growth rate (SGR), Feed conversion ratio (FCR) and Protein efficiency ratio (PER) were calculated following the equation as cited by Pechsiri and Yakupitiyage (2005) [45]. The equations are as follows:
- Specific growth rate (SGR%) = \( [(\text{ln final weight – ln initial weight})/\text{time}(\text{days})] \times 100 \)
- Feed conversion ratio (FCR) = total amount (g) of dry feed supplied/wet weight (g) gain of fish
- Protein efficiency ratio (PER) = Weight gain (g)/protein consumed (g)

Statistical analyses were done using Microsoft Office Excel and SPSS (Statistical Package for Social Science) software.

Results
Physicochemical characteristics
Throughout 105 days of culture water quality in all ponds were observed to be normal and remained within ranges which allow high growth rate and production of Nile tilapia (Fig. 1). Temperature of water was 29-34°C and almost same in all ponds. Depth of water was maintained at a level of one...
meter in all ponds. As shown in Fig. 1a, salinity of water was also almost same in all ponds. Salinity of water was 12 ppt during stocking and increased to highest level 16.5 ppt at 55-65 days of culture and again gradually decreased to 10 ppt at the later part of the culture period. Transparency of water was initially higher in all ponds and gradually decreased with the progress of culture period (Fig. 1b). pH of water of all the ponds is congenial for culture and varied from 7.7-9.0 (Fig. 1c). Alkalinity was almost same (116-155 mg/l) in all ponds (Fig. 1d).

Fig 1: Variation in some water quality parameters of the experimental ponds

Initial level of morning dissolved oxygen (DO) was 5.0-6.70 mg/l which decreased to 1.2 at the later part of the culture period (Fig. 1e). At this level of low DO, no mortality of tilapia was observed.

**Bacterial count**
Total Heterotrophic Bacterial population was higher in probiotics treated ponds in comparison to that of control pond and highest bacterial count was observed in both water and soil of pond where tilapia was fed with safegut treated feed but there was no significant difference among treatments (Table 2). The highest pathogenic bacteria (Vibrio sp.) in both water and soil of pond were found in control pond but there was no significant difference among treatments.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Probiotics</th>
<th>Water</th>
<th>Soil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>THB (CFU/ml) x 10^4</td>
<td>Vibrio sp. (CFU/ml) x 10^4</td>
</tr>
<tr>
<td>T1 Safegut</td>
<td>1.65±0.494</td>
<td>0.017±0.002</td>
<td>52.75±10.253</td>
</tr>
<tr>
<td>T2 Zymetin</td>
<td>1.3±0.282</td>
<td>0.014±0.001</td>
<td>49.85±8.273</td>
</tr>
<tr>
<td>T3 Probio-Aqua</td>
<td>1.5±0.565</td>
<td>0.014±0.002</td>
<td>58.25±10.960</td>
</tr>
<tr>
<td>T4 No probiotics</td>
<td>1.15±0.212</td>
<td>0.019±0.003</td>
<td>47.1±6.505</td>
</tr>
</tbody>
</table>

**Growth and production of fish**
The growth performance and feed utilization of *O. niloticus* fed with different probiotics as dietary supplementation are given in Table 3. The highest production was obtained at T3, where tilapia was supplied with Probio-aqua treated diet, whereas the control diet produced the lowest production. But there were no significant (*P*<0.05) different in production among four treatments. Results show that, the highest survival was found in T3 having a significant different with control (T4) and T1, but no significant (*P*<0.05) different with T2. Lowest survival was found in T1 having no significant different with control (T4). SGR and PER did not display any significant (*P*<0.05) difference among the four treatments. Highest SGR was obtained at T1, where tilapia was supplied with Safegut treated feed. The lowest FCR was obtained at Probio-aqua supplemented diet and significantly different from FCR by control diet, but not significantly different from FCR by Safegut and Zymetin supplemented diet. Fish fed the control diet consumed more diet giving the highest FCR and lowest PER.
The exact mode of action of the probiotic has not been fully elucidated and there is continuous argument about its effect on the water quality. In the present study, there is no obvious effect of the probiotics added to feeds on water quality, this agrees with the findings of Yanbo and Zirong (2006) [61].

In the present study, production was higher in all probiotics supplemented ponds than control ponds which similar to the findings of Noh et al. (1994) [40]; Bogut et al. (1998) [9] and Nikoskelaine et al. (2001) [30] who obtained better growth response with diets supplemented with probiotics containing bacteria. Since the first use of probiotics in aquaculture, a growing number of studies have demonstrated their ability to increase the growth rate and welfare of farmed aquatic animals Lara-Flores et al. 2003 [39]; Bligh and Dyer, 1959 [8]; Macey and Coyne, 2005 [44]; Wang et al, 2005 [50], 2006 [51], 2007 [52]. Despite that, Shelby et al. (2006) [53] and He et al. (2009) [54] revealed that growth performances of tilapia were not significantly improved by dietary supplementation of yeast (S. cerevisiae) at different levels. In the present study, the highest FCR and the lowest PER was found in control ponds no probiotics was used. These indicate that, fish fed control diet consumed more diet than the other treatments. Evidence is available that indicates gastrointestinal bacteria take part in the decomposition of nutrients, provide the macroorganisms with physiologically active materials Bairagi et al, 2002 [5], 2004 [4]; Ramirez and Dixon, 2003 [46]; Sugita et al, 1992 [55], 1997 [56], Wang and Xu, 2006 [57]; Wang, 2007 [58]; Ai et al, 2011 [2] and thus facilitate feed utilization and digestion. This may account for the enhanced PER by dietary supplementation of probiotics in the present study.

The addition of probiotic as live supplements in the diet allows probiotic to survive passage through the intestinal tract (Fuller, 1992) [21]. Microorganisms and their enzymes have an important role in the digestion process (Murilla-Moran et al., 1990) [30] by increasing the total enzyme activity of the gut (Ding et al., 2004; Ziaei-Nejad et al., 2006) [15, 63] and stimulating the production of endogenous enzymes (Ochoa-Salano and Olmos-Soto, 2006; Wang, 2007) [41, 58] which in turn can increase the food digestibility. In addition, the exogenous enzymes have a broader pH range than endogenous enzymes that prolongs the digestion period and may allow better hydrolysis of substrates. As pointed by several authors the digestive enzymes (amylase, protease and lipase) could be improved by administration of probiotics to the diet (Ziaei-Nejad et al., 2006; Wang, 2007) [63, 58].

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### Table 3: Mean and standard deviations of average body weight (ABW), survival (S), production (P), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) of tilapia in different treatments after 105 days of culture.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>ABW (g)</th>
<th>S%</th>
<th>P (kg/ha)</th>
<th>SGR%</th>
<th>DWG (g)</th>
<th>FCR</th>
<th>PER</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 Safeguard</td>
<td>242.6±43.73a</td>
<td>66.02±2.38a</td>
<td>8007±165.46</td>
<td>7.04±0.01</td>
<td>2.31±0.03a</td>
<td>1.50±0.07ab</td>
<td>2.38±0.12</td>
</tr>
<tr>
<td>T2 Zymetin</td>
<td>183.73±4.26b</td>
<td>89.64±3.58b</td>
<td>8241±728.32</td>
<td>6.77±0.02</td>
<td>1.75±0.02b</td>
<td>1.24±0.01a</td>
<td>2.88±0.03</td>
</tr>
<tr>
<td>T3 Probio-Aqua</td>
<td>187.98±5.32b</td>
<td>92.35±6.41b</td>
<td>8681±626.50</td>
<td>6.79±0.01</td>
<td>1.78±0.01b</td>
<td>1.23±0.04a</td>
<td>2.90±0.09</td>
</tr>
<tr>
<td>T4 No probiotics</td>
<td>203.26±19.79ab</td>
<td>68.40±3a</td>
<td>6967±982.17</td>
<td>6.86±0.09</td>
<td>1.93±0.19ab</td>
<td>1.56±0.04ab</td>
<td>2.29±0.09</td>
</tr>
</tbody>
</table>

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