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

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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/m^2. 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*, *Streptcoccus*, *Weisslla*) and gram negative bacteria (*Aeromonas*, *Alteromonas*, *Photorhodobacterium*, *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 Gatesoupe, 1998 [47]; 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 (Nageswara 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 (*Oreochromis 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].

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) [27] 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) [35] 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 m^2 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 1: Experimental design

Treatments	Probiotics	Mode of application	Stocking density (No/m^2)	Replications
T1	Safegut (*Bacillus subtilis, B. licheniformis, L. sporogrnrs, S. boulardii, S cerevisiae, Asdpergillus oryzae, A. niger,* Vit. B1 & B6, coated vit. C and some enzymes)	Dietary supplementation	5	2
T2	Zymetin (*Bacillus mesentericus, Streptococcus faecalis, Clostridium butyricum*)			
T3	Probio-Aqua (*Rhodopseudomonas palustris* & some basic media)			
T4	No probiotics (control)			

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 m^2) was prepared at one corner in each pond by erecting nylon net fastened in bamboo frame. All ponds were filled with 11 ppt tidal water of Shibsa river up to a depth of one meter after filtering through nylon net. Water of the ponds was treated with rotenone @ 2 ppm to kill unwanted and predatory fishes. Dolomite @ 20 ppm was applied to each pond to increase buffer capacity of the ponds. After three days, water of the ponds was fertilized with urea and TSP @ 2.5 ppm and 3.0 ppm, respectively. After growth of sufficient plankton, fries (ABW, 0.15g) of male Nile tilapia, *O. niloticus* were stocked uniformly to the in-pond nursery of each pond. The procured fries were produced and transported in freshwater. Before stocking, fries were acclimatized to the pond water gradually for one hour.

Feeding Trial

In the nursery, the stocked fries of tilapia were fed with commercial nursery feed (containing 35% protein) @ 20% of total tilapia biomass. After 15 days of nursing, fishes were released to the whole pond by up-folding the nylon net of the nursery. At this stage, fishes were fed with floating crumble feed (28% protein) @ 10% of total fish biomass. The feeding rate was gradually reduced to 3.0% at the end of the culture period. Growth of fishes was monitored at weekly interval and feed was adjusted accordingly. Daily diet was fed thrice a day. Feed was supplemented with probiotics @ 5g or ml per kg feed according to the experimental design as given in Table 1. Feeds and probiotics powder were soaked with water and mixed together, then it was air dried about 30 minutes and distributed in water. After 85 days of culture, 20% water of all ponds was exchanged with tidal water to reduce the organic load of the ponds.

Water quality parameters

Water quality parameters *viz.,* temperature, depth, salinity, pH, transparency and total alkalinity were determined at weekly intervals and dissolved oxygen (DO) was determined 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.

Bacterial count (CFU/ml) = CFU × Dilution Factor

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%) = [(ln final weight – ln initial weight)/time(days)] x 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 29-34ºC 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).

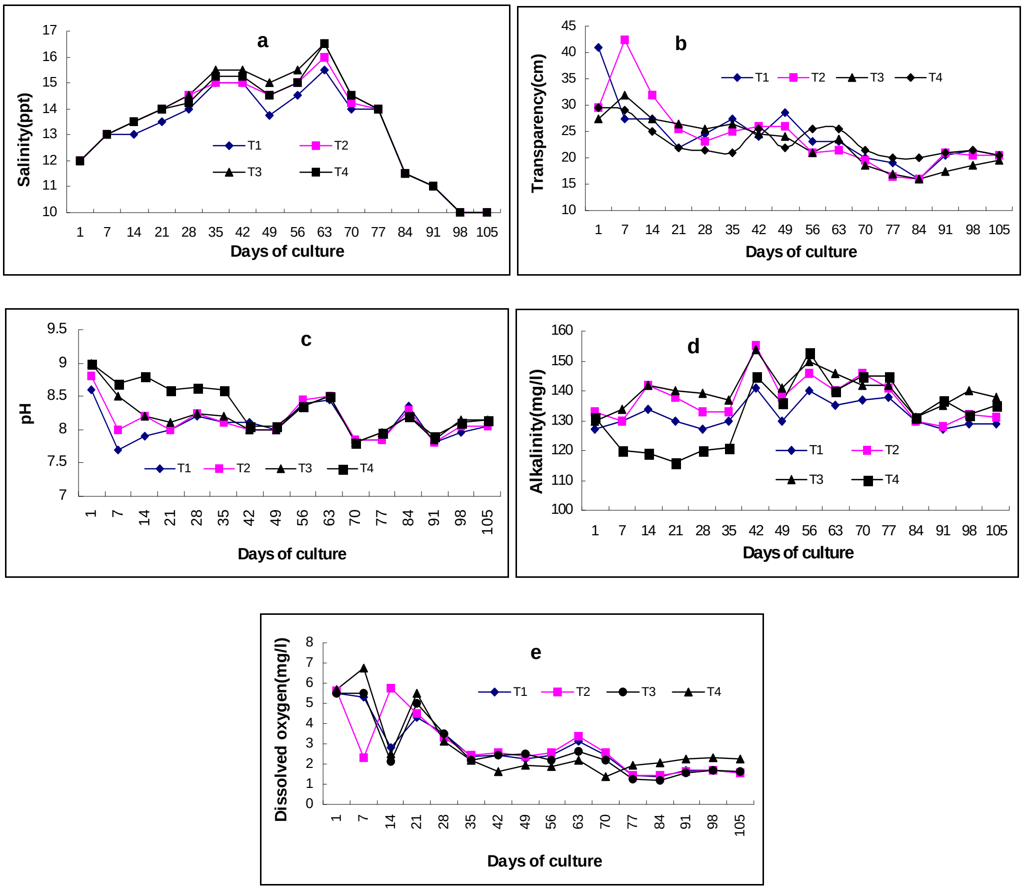

Fig 1: Variation in some water quality parameters of the experimental ponds

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 2: Mean and standard deviations of total heterotrophic bacteria (THB) and *Vibrio sp.* in different treatments.

Treatments	Probiotics	Water		Soil	
		THB (CFU/ml) x 10^4	*Vibrio sp.* (CFU/ml) x 10^4	THB (CFU/g) x 10^4	*Vibrio sp.* (CFU/g) x 10^4
T1	Safegut	1.65±0.494	0.017±0.002	52.75±10.253	0.048±0.009
T2	Zymetin	1.3±0.282	0.014±0.001	49.85±8.273	0.047±0.007
T3	Probio-Aqua	1.5±0.565	0.014±0.002	58.25±10.960	0.041±0.005
T4	No probiotics	1.15±0.212	0.019±0.003	47.1±6.505	0.052±0.004

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.

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.

Treatments	ABW(g)	S%	P (kg/ha)	SGR%	DWG(g)	FCR	PER
T1 Safegut	242.64±3.73[a]	66.02±2.38[a]	8007±165.46	7.04±0.01	2.31±0.03[a]	1.50±0.07[ab]	2.38±0.12
T2 Zymetin	183.73±4.26[b]	89.64±5.85[b]	8241±728.32	6.77±0.02	1.75±0.02[b]	1.24±0.01[a]	2.88±0.03
T3 Probio-Aqua	187.98±0.53[b]	92.35±6.41[b]	8681±626.50	6.79±0.01	1.785±0.01[b]	1.23±0.04[a]	2.90±0.09
T4 No probiotics	203.26±19.79[ab]	68.40±3[a]	6967±982.17	6.86±0.09	1.935±0.19[ab]	1.56±0.04[b]	2.29±0.09

Discussion

The exact mode of action of the probiotic has not been fully elucidated and there is continuous argue 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 finding 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) [39] 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 [30]; Bligh and Dyer, 1959 [8]; Macey and Coyne, 2005 [34]; Wang *et al*, 2005 [59], 2006 [57], 2007 [58]. Despite that, Shelby *et al.* (2006) [50] and He *et al.* (2009) [26] revealed that growth performances of tilapia were not significantly influenced 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 [52], 1997 [53]; 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 di*et al*lows probiotic to survive passage through the intestinal tract (Fuller, 1992) [21]. Microorganisms and their enzymes have an important role in the digestion process (Munilla-Moran *et al*., 1990) [36] 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].

Conclusion

From this study it can be concluded that, probiotics supplemented with diet have no effect on brackish water pond environment. Oral probiotics have some positive effect on growth, survival and production of Nile tilapia but not significantly affect on overall production in brackish water pond.

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