Journal of Aquaculture
Research & Development

Rahman et al., J Aquac Res Development 2019, 10:2
DOI: 10.4172/2155-9546.1000564

Research Article

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Influence of Probiotics on the Growth Performance of Sex Reversed Nile Tilapia (*Oreochromis niloticus*, Linnaeus, 1758) Fry

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Abstract

Tilapia culture could contribute a lot in boosting the production of fish so current investigation was aimed to improve the growth, feed utilization, survival of Nile Tilapia (*Oreochromis niloticus*) fry and benefit cost ratio by using probiotics during sex reversal and other steps of culture. The investigation was designed in three treatments where the first one fed on diet supplemented with *Bacillus* sp., and the second fed on diet containing *Lactobacillus* sp. while the third treatment fed on basal diet which was considered as control. After 100 days of investigation, it was observed that the fish groups fed with probiotics supplemented diets revealed significant improvement in aspect of growth. Highest yield of fingerling 3.4 kg was found in the treatment fed with *Bacillus* sp. which ultimately influence the gross revenue. Fry survivality was also found higher upto 66% in the treatments fed with probiotics compared to the control where there was no probiotic added in the feed. In all the treatments sex reversal rate was found the same (97%), revealed that the addition of probiotics in fish feed improves growth and survival of the sex reversed fingerling. Therefore, these probiotics might be useful to get higher production of sex reversed fish.

Keywords: Nile tilapia; *Oreochromis niloticus*; Growth performance; Probiotic; Sex reversal fry; *Bacillus; Lactobacillus*

Introduction

In the present world, fish culture is increasing day by day to mitigate the shortage of animal protein [1]. Provisions of suitable environmental and growth conditions are necessary for proper growth of fish to get increased production. Hence fry source is much important for successful fish farming. The cost of the fry considered as an important factor in overall production costs of aquaculture and efforts should made to find new insights of seed production to increase the profitability of the aquaculture farm.

Fish production is being gradually increased through aquaculture in the closed waterbodies, but the increasing trend of fish production is lesser than that of population boom. So, it is strongly felt that all sorts of efforts need to be employed to increase the fish production to fulfill the protein demand of the people. So, the development of culture system for the rapid growing species is the prime concern to increase fish production.

Improved biological management is a priority to develop a suitable culturable fish species that will reduce the declination of resources and enhance fish productions. Tilapia is the second most farmed fish world-wide and represents about 42% of the world total fish production [2] and its production has quadrupled over the past decade because of its suitability for aquaculture, marketability and stable market prices [3]. Tilapia is considered as an important exotic species which is fast grower, suitable for culture in our native waterbody, has high tolerances of environmental pollution and have been named as the "Aquatic chicken" [4]. Tilapia proved its importance as the aquaculture species of the 21st century [5]. Tilapia can contribute a lot in boosting the production of fish without increasing the operational costs.

Precocious maturity and uncontrolled reproduction pattern limited the production of Tilapia [6]. Under ideal condition female fish are capable of spawning every four to six weeks in natural environment [7] which leads to overcrowding, competition for food and stunted the growth and resulted in low production. Several methods have been proposed and developed and applied to overcome this problem. Use of suitable predator species [8], the generation of infertile fry through triploid and the use of hybrid crosses to produce monosex broods [9,10] are few of them. Monosex population offers benefits, including rapid growth and prevention of unwanted population.

To induce sex inversions of genotypic females into phenotypic males has proven to be successful methods to produce monosex population by using male sex steroids [11]. The androgen 17α-methyltestosterone (MT) [12] and the estrogen diethylstilbestrol (DES) are popularly used hormones for sex inversion in Tilapia. The most common method for monosex male production is the direct masculinization of Tilapia using hormone [13,14]. Monosexing facilitated the development of Tilapia farming [15]. To grow and function reproductively as males, male steroid hormone is administered to first feeding fry; hence the undifferentiated gonadal tissue of genetic females develops into testicular tissue.

Stocking of high density of fish seed is a prerequisite for commercial aquaculture which leads diseases caused by various types of stress in the steps of cultivation, resulted in higher mortality rate and economic losses. To minimize these problems antibiotics has been used in aquaculture [16]. Most utilized growth promoting additives includes hormones, antibiotics and some salts [17]. Although these do promote growth, their improper use can result in adverse effects in the animal and the final consumer and can lead to resistance of pathogenic bacteria in case of antibiotics [18]. An alternative to the antibiotics is the use of beneficial bacteria (probiotic) to fight the pathogenic bacteria by competitive exclusion, which is an acceptable practice in animal

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Received January 17, 2019; **Accepted** February 21, 2019; **Published** February 28, 2019

Citation: Rahman Z, Mamun A, Ahmad I, Rashid I (2019) Influence of Probiotics on the Growth Performance of Sex Reversed Nile Tilapia (*Oreochromis niloticus*, Linnaeus, 1758) Fry. J Aquac Res Development 10: 564. doi: 10.4172/2155-9546.1000564

husbandry [19]. On the other hand, similar to other fish farming, Tilapia culture has also been facing economic losses because of mortality during the sex reversal of fry.

By considering the above all factors, the use of probiotics as an alternative strategy has been receiving increasing attention world-wide in Tilapia culture. Probiotics offer a promising alternative [20] to assist in the protection of aquacultured species from diseases. As probiotics improve fish growth and modify internal microbial community, use probiotic either alone or in combination with prebiotics is recently the goal of the disease biocontrol strategy in aquaculture [21].

Probiotics alter the microflora in intestine of the host and exert beneficial health effects in host [22]. Probiotics live in the gastrointestinal tract of the host, adhering to the epithelial wall and proliferating in the intestine; improve adhesion capacity to the intestinal mucosa that hindered the adherence of pathogenic bacteria. The probiotics play in bioremediation and assisting in digestion by producing digestive enzymes and improved feed utilization and immune system. In aquaculture industry, several probiotic species were used, including *Saccharomyces* spp. [23], *Lactobacillus acidopilus* [24], *B. subtilis* [25-27] and mixed cultures [28].

The exact role of probiotics is yet to be understood; probiotics often demonstrate different role against specific host and specific strain. Probiotics increases growth, improves efficiency and stimulate pre-digestion of anti-nutritional factors present in the ingredients [29]. Indeed, many investigations about probiotics have been done on aquaculture; there are fewer publications on role of probiotics in tilapia fry production and sex reversal. Effects of probiotics in aquaculture deserve important highlight on the economic evaluation, by which farmer can get profit by producing quality fish. *Bacillus* sp. and *Lactobacillus* sp. have been recently used as probiotics in Tilapia [30-34].

The *Bacillus* sp. and *Lactobacillus* sp. are great candidates as probiotics used in aquatic animals because they are capable to survive in high temperatures [35] such as after the pelleting process of feed. This feed can be stored at room temperature without any deleterious effect and resisted to the low pH that can reach intact to the small intestine [36].

The present investigation was conducted to understand the effects of different probiotics (*Bacillus* sp. and *Lactobacillus* sp.) on the growth response, feed utilization, sex reversal and survival in sex reversed Nile Tilapia (*Oreochromis niloticus*) fingerling production.

Materials and Methods

Experimental design

The experiment was carried out for a period of 100 days to determine the effects of probiotics on the growth response, feed utilization, and survival of Nile Tilapia (*O. niloticus*) fry during the sex reversal phase in the circular tank. A completely randomized design (CRD) [37] was established with 3 treatments (T-I, T-II and T-III) each with three replications. Each treatment contained 250 spawns. The average length and weight of spawns were 7.75±0.05 mm and 0.006 g, respectively and stocking density was 10 spawns/l of water. The spawns were reared for 30 days in 25 liter (sex reversal phase) and another 75 days in 125-liter plastic tank (fingerling phase). In T-I Micro guard (Zeus Biotech Ltd. India), in T-II Probac (Nuvista Pharma Ltd. Singapore) was provided along with basal feed. In T-III basal feed was provided without any probiotic. Synthetic androgen 17 α-methyltestosterone (MT) was mixed with feed (60 mg MT/kg Feed) by volatilization of ethanol and fed during the sex reversal phase. Micro guard contains *Bacillus* sp mainly comprise with *Bacillus subtilis* and Probac contains the *Lactobacillus* sp.

Experimental Fish

The Nile Tilapia (*O. niloticus*) fry was collected from a renowned commercial hatchery. The fry were transported and gently released into the acclimatization tank with sufficient aeration. After acclimatization fry were transferred to experimental tanks.

Diet formulations

Feed with 60 mg/kg of MT hormone were prepared by ethanol evaporation method [38]. To prepare 100 g feed for each treatment; required amount of MT hormone (6 mg) was diluted with 60 ml of alcohol (Merck) for homogenous mixing with the commercial feed (Aftab Fish Feed) in each treatment. It contained 30% crude protein, 3.7 Kcal/g of metabolizable energy, 3.4% fiber and 7.03% fat as well as vitamins and minerals in the form of dry pellets. Probiotic containing 5.0 $\times 10^8$ cfu per gram Micro guard and Probac was used as growth promoters. In the feed, probiotic was added 1% per 100 g feed when the ethanol was evaporated. The probiotic was added to the diet for the experimental test during the process of pelletization.

Process of diet formulation and preservation

The required amount of hormones and fish meal was measured by electrical balance (Shimadzu). MT hormone was diluted with ethanol, poured into the plastic container which was already filled with starter feed. The feed was then mixed vigorously for homogenous mixing. After that the feed were air dried separately and probiotic was mixed with keeping the lid of the containers open until the feed become fully dried. The feed were then converted into powdery form and preserved in the refrigerator at 4°C for future use.

Rearing of spawns

The spawns were fed with hormone mixed feed 4 times a day up to satiation. The water of each tank was exchanged by 75% of the volume with fresh water once a day in the morning to avoid water quality deterioration due to food wastage and feces of the spawns. In addition, the fecal out-put and wastages of feed were removed from the tank by siphoning daily. Additional oxygen to aquarium was provided through aeration and was stopped each time when fish received the supplied feed.

Sampling of the fish

The fish were sampled at weekly interval to determine the increase in their size in term of length and weight. Sampling was done in the early morning when the fish stomach was about to be empty to avoid the biasness of weight due to the presence of excessive feed. Ten fish were randomly collected from each tank. The weight (mg) was taken in an analytical balance (Shimadzu) and the length (mm) was measured by placing the fish on the petri dish having a 1mm graph paper underneath it. Mortality of the fry was recorded daily. The experiment was continued for 30 days and at the end of the experiment the fish were exposed high water depth in the tank and reared those with the normal feed until being sexed.

Monitoring water quality

Parameters of water quality were determined according to the methods of APHA [39]. Temperatures (digi-thermo WT-2) were recorded daily in each tank. For water analysis, samples from the inflow pipe and fish culture tanks were collected. Dissolved oxygen (DO) and pH were measured using Oxygen and pH meters (Hach Co., Loveland, Colorado).

Survival (%) of the fish

The hormone feeding was terminated at the 30th day of experiment but the fish were reared for another 70 days with normal feed for proper sexing and after the age of 100 days, the final growth and survival (%) of fish were estimated.

- Survival Rate (%)=(Total number of fish harvested/Total number of fish stock) × 100

Growth parameter

The following parameters were used to evaluate fry growth:

- Length gain (mm)=Mean final length-Mean initial length
- Weight gain (g)=Mean final weight-Mean initial weight
- % Weight gain (g)=(Mean final weight - Mean initial weight/ Mean initial weight) ×100
- Specific Growth Rate (%/day)=(Ln WT – Ln Wt/T–t) × 100 Where, Ln WT=natural log of weight at time T1.

Ln Wt=natural log of initial weight.

T= time, t= initial time, Ln-T-I= Natural logarithm [40].

Production of fish

Gross production and net production of fish were measured by following formulae:

- Gross production=No. of fish caught × Average final weight.
- Net production= No. of fish caught × Average weight gained.

Feed utilization

Feed conversion ratio (FCR) was measured as:

- FCR=total Feed consumed by fish (g)/Total weight gain by fish (g) [41].

Benefit cost ratio

The following formula was used to measure the benefit cost ratio:

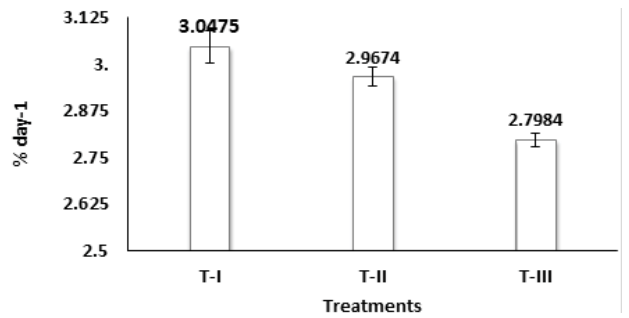
- BCR= Gross Income/cost
- Gross income=(Quantity of Main product x price of Main product)+(Quantity of by product x price of by product)
- Cost=Expenses incurred for experiment [42]

Fish sexing

The fish was sexed by gonad squashing and acetocarmine staining method [14]. The fish was killed and the viscera was removed to reveal the two thread like gonads lying along the surface of the body cavity on either side of the kidney (Figures 1-4). The gonads were removed and placed on a clean glass slide. A few drops of acetocarmine stain were added and the gonads squashed with a cover slip. The sex of the fish was identified by examining the slides under a microscope. The male gonad is composed of fine granular like structure of spermatogonia (Figure 5) and the female is characterized with the structure of circular oogonia (Figure 5a).

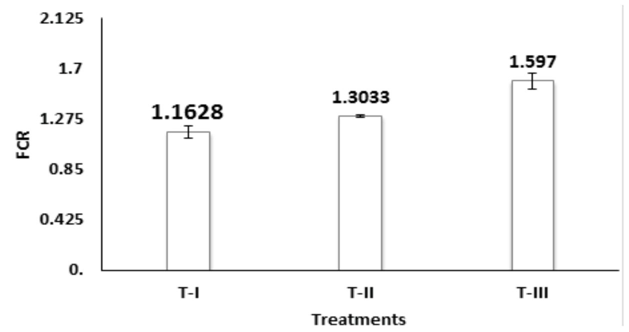
Data analysis

During experimental period all the data were collected, recorded and preserved on computer spreadsheet. The data were analyzed statistically by one-way analysis of variance ANOVA using statistical software. Means were given with ± standard deviation (SD).



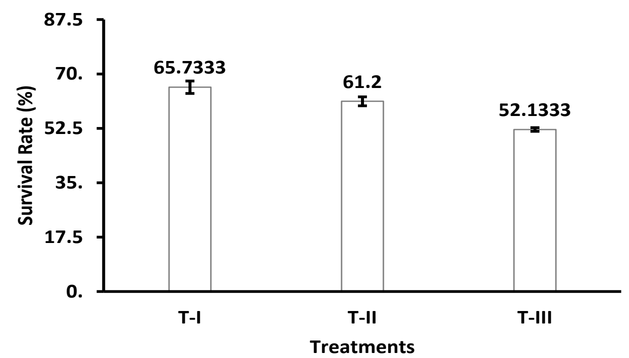
***Means for each treatment are significantly different (p<0.05).

Figure 1: Specific growth rate of Nile tilapia (*O. niloticus*) fed with different probiotics during the experiment.



***Means for each treatment are significantly different (p<0.05).

Figure 2: Feed conversion ratio of Nile tilapia (*O. niloticus*) fed with different probiotics.



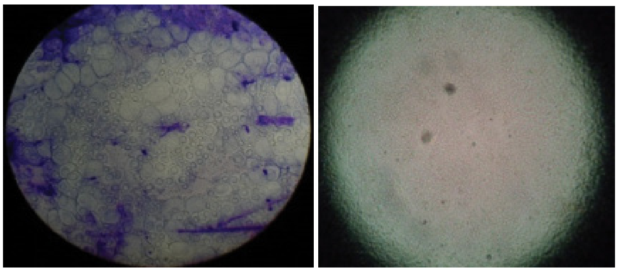
Means for each treatment are significantly different (p<0.05).

Figure 3: Survival rate (%) of Nile tilapia *(O. niloticus*) fed with different probiotics during the experiment.

Results

The pH, temperature (°C) and other rearing conditions were maintained the same in all the treatments (Table 1). There were no significant differences found in water quality parameters among the treatments. Range of water temperature was observed between 27°C to 29°C. Furthermore, the Dissolved oxygen (DO) level was found between 7.28 mg/l and 7.8 mg/l and pH level was 7.3 to 7.4.

Figure 4: Finding and removing the gonad from a Nile tilapia *(O. niloticus)* fingerling.



(a) (b)

Figure 5: Ovarian (left) and testicular (right) tissue of Nile tilapia *(O. niloticus)* fingerling as they appear in a gonadal squash.

The average initial length and average initial weight was found 10.13 ± 0.12 mm and 0.021g, respectively (Tables 2 and 3). During sampling at 7th, 14th, 21st, and 28th days, among the treatments there were no significant variations observed in length and weight of fry. At the end of the experimental period it was observed that the both treatments fed probiotics supplemented diets revealed significant impact in the body weight (BW) and body length (BL). BL gain was found highest (63.13 ± 1.22 cm) in T-I, where *Bacillus* sp. was added. When *Lactobacillus* sp. was used the average BL gain was found 53.28 ± 1.12 cm. In control, where there was no probiotic added in the diet, the BL was found 46.00 ± 0.43 cm which is lowest among all the treatments (Table 2). Final BW was found the highest in T-I. Use of *Lactobacillus* sp. influences the growth of the fry and BW was found higher than the control (Table 3).

In the present investigation, specific growth rate (SGR) represented the relationship between the body weight and length of Nile Tiilapia (*O. Niloticus*) fed with different probiotics was found the highest (3.05 ± 0.04) in the treatment fed with *Bacillus* sp. compared to other treatments (Figure 1). Moreover, feed conversion ratio (FCR) of Nile Tiilapia (*O. Niloticus*) fed with *Bacillus* sp. was found lowest (1.16 ± 0.04) in T-I. The highest (1.59 ± 0.07) FCR was found in the control where there was no probiotic added in the diet (Figure 2).

A high rate of mortality was observed in all the treatments and in first 12 days of experiment, comparatively more mortality was observed (Figure 3). The initial survivability was found to be lower in all the treatments, but survivability increased with the advancement of experimental time. At the end of the experiment, comparatively more fish was found to survive in T-I than those of all other treatments. The cumulative survivability in all the treatments was moderate at the end of the experiment. Using 17 α-methyltestosterone fish were sexed at the age of 100 day. The result of sex ratio is found similar (97%) in all the treatments. Benefit cost ratio (BCR) was found higher (2.33 ± 0.06) in T-I, followed by T-II (2.02 ± 0.05) and lowest (1.70±0.03) was observed in T-III (Table 4.)

The gross yield of fry obtained from all the treatments ranged from 21.51 to 34.66 kg with the highest yield in T-I and the lowest yield in T-III (Table 4). Profit of tilapia farming as obtained in the present investigation was the highest (BDT. 752.42) in T-I followed by T-II (BDT. 644.20). The lowest profit (BDT. 585.17) was found in control. BCR was also observed highest in T-III (1.61) followed by T-II (1.43) and T-III (1.19) (Table 4).

Discussion

Measurement of water quality parameters is a pre-requisite for maintaining a healthy aquatic environment and better production for aquatic organisms. There was no significant ($p<0.05$) difference found in water quality parameters among the treatments during the whole experimental period and was found within the optimum levels recommended for fishes [43] indicating that, the experimental diets had no detrimental effects on the surrounding water quality. The range of water temperature of the present study was observed 27-29°C whereas the average pH level was found 7.4, indicates that it is suitable for fish

Parameters	Value
Temperature (°C)	28 ± 2
pH	7.3–7.6
Dissolved oxygen (mg/l)	6.8-7.5

Table 1: Water quality parameters observed in the experiment.

Sampling Days	Average length (mm)		
	T-I	T-II	T-III
Day 0	10.11 ± 0.12	10.13 ± 0.12	10.1 ± 0.12
Day 7	10.82 ± 0.35	10.82 ± 0.35	11.5 ± 0.43
Day 14	14.33 ± 0.28	13.83 ± 0.76	12.33 ± 0.38
Day 21	20.3 ± 0.26	17.63 ± 0.32	14.25 ± 0.25
Day 28	26 ± 0.5	23.92 ± 0.72	19.33 ± 0.76
Day 100	73.26 ± 1.23	63.42 ± 1.01	56.58 ± 0.38
Length gain	63.13 ± 1.22 [a]	53.28 ± 1.12 [b]	46.00 ± 0.43 [c]

*The fry were fed up to 100 days with basal feed and probiotics after the end of hormonal feeding in different treatments.
**Means followed by the different letters in each column for each treatment are significantly different ($p<0.05$).

Table 2: Growth in length (mm) of Nile tilapia fry (*O. niloticus*) during the experimental feeding period.

Sampling Days	Average Weight (g)		
	T-I	T-II	T-III
Day 0	0.02	0.02	0.02
Day 7	0.03	0.03	0.03
Day 14	0.04	0.04	0.03
Day 21	0.05	0.05	0.05
Day 28	0.09	0.06	0.05
Day 100	21.09 ± 0.92	19.46 ± 0.49	16.51 ± 0.30
Weight gain(g)	21.07 ± 0.92 [a]	19.44 ± 0.49 [b]	16.42 ± 0.07 [c]

*During sampling at 7 days interval, weight of 10 fry were measured together, therefore it was not possible to calculate the standard deviation.
**The fry were fed up to 100 days with normal feed with probiotic after the end of hormonal feeding in different treatments.
***Means followed by the different letters in each column for each treatment are significantly different ($p<0.05$).

Table 3: Growth in weight (g) of Nile tilapia fry (*O. niloticus*) during the experimental feeding period.

Parameters	Treatments		
	T-I	T-II	T-III
Survival	164.33[a]	153.00[b]	130.33[c]
Yield (kg)	3.4 ± 1.4[a]	2.9 ± 1.2[b]	2.1 ± 0.75[c]
Price per fry (BDT)	8	7	6
Gross revenue	1314.7[a]	1224.0[b]	1042.7[c]
Total cost (BDT)	562.25[a]	529.80[b]	457.50[c]
Net return (BDT)	752.42[a]	694.20[b]	585.17[c]
Feed (kg)	4.03[a]	3.88[b]	3.43[c]
Price of feed (BDT)	362.25	329.80	257.50
BCR	2.34 ± 0.06[a]	2.02 ± 0.05[b]	1.71 ± 0.03[c]

Means followed by the different letters in each column for each treatment are significantly different (p<0.05).

Table 4: Economic analysis in the cost of production of Nile tilapia (*O. niloticus*) during feeding different probiotics.

culture [44,45]. Artificial aeration was provided in each of the rearing tanks and therefore, DO level did not differ significantly ($p<0.05$). The DO content (5.0 to 7.0 ppm) of water considered to be fair or good in respect of productivity [44]. According to FAO the range of suitable DO for fish culture would be 5-8 mg/l [46] which recommended that the level of DO in the present study was within the productive range and suitable for fish culture.

During sampling on 7th, 14th, 21st, and 28th days of experiment no significant variations in BL and BW of fry was found though different probiotics were administered. When the fish were sampled at day 100th significant variations in BL and BW of fry was observed (Table 3). The highest BL and BW were found when the fry fed probiotic *Bacillus* sp with the basal diet, compared to other treatments (Table 2). After 61 days of feeding of the normal diet, the *B. amyloliquefaciens* fish showed significantly superior growth and better FCR than the control [45]. Nile Tilapia (*O. Niloticus*) fry and juveniles fed diets supplemented with yeast *Saccharomyces cerevisiae* showed increase in the values of weight [47]. *Bacillus* sp increases the colonization rates in digestive tracts and the specific activities of enzymes was found to exhibit significant increases in survival rate and weight gain as compared to control [48]. The biomaterial Lycogen™ found to increased muscle weight, weight gain and length gain of seawater red Tilapia (*O. mossambicus* × *O. niloticus*) [49]. The BW and WG of Tilapia significantly increased when the diets were supplemented by 0.2% Biogen® [50]. Therefore, it may be stated that the probiotic was effective and consequently, the fish presented better development than in the other treatments, indicating that the use of probiotic is beneficial for the production of Tilapia fry.

Addition of *Bacillus* sp. as probiotic could be responsible for the highest SGR in T-I. *B. subtilis* are products approved by the European Food Safety Authority (EFSA) and supplementation of dietary *B. subtilis* C-3102 improved the gut bacterial content. As the total amounts of viable bacteria increased, it increased up regulation of intestinal enzyme expression and increases the SGR of fish [51]. Addition of probiotic *B. subtilis* C-3102 got the SGR which is almost similar to the present experiment [42]. T-II got the lower SGR (2.96 ± 0.03) which also followed the almost same result that the fish fed *B. cereus* or *B. subtilis* showed a higher SGR of fingerlings compared to those that fed with basal diet only.

The increased growth rate indicated a positive effect of the used probiotic, specially *Bacillus*. The obtained results could be attributed to the ability of *Bacillus* to improve the intestinal fauna which produce wide range of digestive enzymes which let the digest diets effectively Addition of probiotics enhance the ability to detoxify the potentially harmful components of feed and the ability to produce a lot of essential vitamin which resulted in high food utilization. *B. subtilis* was used in the food of common snook (*Centropomus undecimalis*) and observed that these probiotics increased the food absorption and gave a better growth consequently [52]. Also Biogen® as food additive containing *B. subtilis* as probiotic was used to germinate in the fish intestine, which exerted beneficial effects including higher growth rate and higher feed efficiency [35]. Enhancement of the population of beneficial microorganisms consequently improves the digestibility and absorption of food and feed utilization of cultured fish [53].

Fish that have the lower the FCR are considered efficient user of feed. Generally, Tilapia got the FCR 1.6 to 1.8 [48]. T-I got the lower FCR (1.16 ± 0.05) means better users of feed. The biomaterial Lycogen™ increased the FCR of seawater red Tilapia (*O. mossambicus* × *O. niloticus*) [49]. The best FCR values observed with probiotic-supplemented diets suggested that, the addition of probiotics improved feed utilization which indicates that the use of probiotic can decrease the amount of total feed and ultimately reduce aquaculture production cost [54].

The adherence capacity of *B. subtilis* to the intestinal mucosa prevents the disease occurrence [55]. The similar effects of other immune stimulants in aquaculture, probiotics have proved to elevate health status, growth performance, reduce malformations, and improve gut morphology and microbial balance [56]. The initial survivability was found to be lower in all the treatments, but survivability increased with the advancement of experimental time. At the end of the experiment, comparatively more fish survived in the T-I than those of other treatments. The cause of high mortality in the treatments could not be understood. High mortality in both hormone and non-hormone treatments was reported [38] and it is hard to predict any negative impact of hormone on Tilapia survival. The cumulative survivability in all the treatments was moderate at the end of the experiment (Figure 4). The use of *Bacillus* exhibited significant ($p<0.05$) increased in survival (11–17% higher) of fingerlings [48]. A diet supplemented with *L. acidophilus* increased survival rate of Nile Tilapia during a challenge with *A. hydrophila* [57]. The survival values were greater in the fish fed, probiotic *B. cereus* var. toyoi or *B. subtilis* C-3102 in relation to the group fed probiotic free [42], which supports the present study (Figure 3).

Superiority of *B. subtilis* in survival, growth and health status may be due to its effect as biocontrol or bacterial antagonism effect and production of antimicrobial agents. Moreover, stimulation of the immune system using probiotic strains has been reported [58]. The substitution of pathogen by the beneficial population has been considered to be important by many authors [59-61]. Addition of 0.3-0.4% Biogen® to the basal diet increased the survival rate of Tilapia compared with the control [62]. Incorporation of probiotics (*B. subtilis*) in fish diets also significantly increased survival and decreased fry mortality [63].

In the present experiment fish sex was reversed due to addition of 60 mg/kg of 17 α-methyltestosterone. Fish from three treatments were sexed at the age of 100 days. All the treatments showed 97.77% male sex. Like the present study, using *B. subtilis*, *B. pumilus* and *B. lincheniformis* as probiotic in diets for red Tilapia fingerlings, found similar results where probiotics did not influenced the sex reversal of Tilapia [42]. Masculinization of Nile Tilapia (*O. Niloticus*) fry could be unsuccessful if the fry failed to attain a standard length of 12 mm at the end of hormone treatment [45]. All the fry in this experiment attained more or less 12 mm at the end of hormone feeding, might be one of the reason for successful sex reversal.

The gross yield of fry obtained was found highest in T-I and the lowest in T-III. The fish yield of the present study is found similar with the yield reported by Alam et al. [64]. Profit of Tilapia farming as obtained in the present study was observed highest (BDT. 752.42) in T-I followed by T-II (BDT. 644.20). Addition of probiotic *B. subtilis* C-3102 got the highest yield than control and addition of other probiotics [46]. The partial operational costs arising with feed costs showed that, the price of the post-reversed fingerlings was affected significantly among treatments. BCR was found highest in T-I (2.34 ± 0.06) followed by T-II (2.02 ± 0.05) and T-III (1.71 ± 0.03) indicating that highest benefit was obtained from the treatment fed *Bacillus* sp., which may be due to the beneficial bacteria and efficient utilization of the probiotics. This promoted satisfactory return in aquaculture. Evaluating the economic viability of adding probiotic in the Nile Tilapia (*O. Niloticus*)) diet found that use of *B. cereus* showed higher return on investment that the use of *B. subtilis* or the combination *B. cereus* and *B. subtilis*, as part of diets for Tilapias [42].

Supplementation of fish diets with probiotics optimized protein use for growth which could decrease the amount of total amount of feed necessary for fish growth and resulted in reducing production costs which is one of the main objectives of aquaculture. Probiotic diet including *B. subtilis* showed better feed intake and feed utilization compared to control diet. From a nutritional point of view and in agreement with Shelby et al. [65], the present results indicated that the addition of the probiotics in the diet of Nile Tilapia (*O. niloticus*) is recommended to stimulate productive growth performance and nutrient utilization.

Incorporation of *Bacillus* sp. in the aquaculture could improve water quality by influencing the water born microbial population and by reducing the number of pathogens [66]. Further these results could be attributed to the bacteriostatic substances produced by *B. subtilis* and *S. cerevisiae* which improve the growth response, feed utilization, survival of Nile Tilapia (*O. niloticus*) fry and BCR during the sex reversal and fingerling phase in the circular tank.

Based on the obtained results, supplement Tilapia diets with probiotics could be recommended for higher production in aquaculture farming system. Further research is still needed to detect the mode of action of probiotics on digestibility, immune response and stress resistance. Also, it is important to define the probiotic levels administered to fish to avoid over-dosing and under-dosing with resultant lower efficacy and unnecessary costs.

Conclusion

The present investigation was conducted to determine the effects of probiotic on the growth performance of sex reversed Nile Tilapia (*O. niloticus*) fry. Probiotics act as an alternative to chemicals and antibiotics in aquaculture, resulting in huge benefit in better health, growth and survival rates, and producing organic products that are safe for consumers. Probiotic could meet the requirements of sustainable aquaculture development as they can enhance the growth performance and survivability. The inclusion of *Bacillus* sp. and *Lactobacillus* sp. in feed for Nile Tilapia (*O. niloticus*) fingerling improves the growth performance, survival rate and feed conversion ratio without negatively influencing the sex reversal of the fingerlings and BCR. Therefore, it can be recommended in the production of the Nile Tilapia (*O. niloticus*) species during sex reversal and fingerlings phases.

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