Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 28(5): 1781 – 1791 (2024)
www.ejabf.journals.ekb.eg

Effect of Probiotics on Growth Performance, Feed Utilization, and Water Quality Parameters of the Nile Tilapia (*Oreochromis niloticus*) Fingerlings

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ARTICLE INFO

Article History:
Received: July 21, 2024
Accepted: Sept. 29, 2024
Online: Oct. 19, 2024

Keywords:
Nile tilapia,
Oreochromis niloticus,
Probiotics,
Growth performance,
Feed utilization,
Diet

ABSTRACT

This study aimed to investigate the impact of water and feed probiotics on the growth performance, feed utilization and water quality of the Nile tilapia (*Oreochromis niloticus*) fingerlings. Three isonitrogenous (30% dietary crude protein) diets were prepared from commercial components. Five different levels of probiotics were used: P0 (no probiotics), Pd0.5 (0.5g probiotics/kg diet), Pd1.5 (1.5g probiotics/kg diet), Pw0.5 (0.5g probiotics/kg diet + 0.05g probiotics/L water), and Pw1.5 (1.5g probiotics/kg diet + 0.05g probiotics/L water). Rectangular glass aquariums (15 aquariums) were used. The Nile tilapia fingerlings (7g) were transferred to the experimental aquariums at a rate of 10 fingerlings per aquarium. The water quality in the system was daily monitored. One-third of the aquarium water was daily changed in the no-probiotic treatments, while in the treatments with water probiotics, the water remained unchanged during the experiment. The control treatment and all probiotic-treated groups exhibited significantly higher (P< 0.05) final body weights, weight gains, and specific growth rates compared to the Pw0.5 group, with no significant difference between the Pw0.5 and Pw1.5 groups. Additionally, the condition factor showed no significant differences (P> 0.05) among the experimental groups. There were also no significant differences (P> 0.05) in survival rates between the probiotic treatments and the control group. The feed conversion ratio (FCR) and protein efficiency ratio (PER) did not differ significantly among the experimental groups, with the Pw0.5 group achieving the lowest values. The protein productivity value (PPV) in the control group was significantly the highest, while the Pw0.5 group recorded the lowest value, showing no statistical difference from the Pd0.5, Pd1.5, and Pw1.5 groups.

INTRODUCTION

The significance of aquaculture in providing animal protein is evident, especially with the growing human population and the plateauing productivity of capture fishing. Over the past eight years, the output of the Nile tilapia (*Oreochromis niloticus*) has increased by 70%, positively impacting global freshwater aquaculture **(FAO, 2022)**. The Nile tilapia originates from Africa. It is known for its ability to thrive in diverse climate conditions adapting to different farming methods. The Nile tilapia is expected to be increasingly farmed worldwide and is currently the third most cultivated fish species, following silver carp and grass carp. It plays a significant role in the maintenance and economies of rural communities in countries where it is cultivated **(El-Sayed & Fitzsimmons, 2023)**. The growth of aquaculture is facing various challenges as it becomes more intensive and commercialized. These challenges include water quality management, developing appropriate feed and feeding methods, improving and controlling broodstock, fighting and preventing diseases and enhancing hatchery and grow-out technologies **(Subasinghe *et al*., 2003)**. The use of expensive antibiotics and chemotherapy drugs hurts aquatic environments. Therefore, it is progressively important to improve the production of healthy fish by using natural alternatives such as probiotics **(Abdellah *et al*., 2024; Vijayaram *et al*., 2024)**. The utilization of probiotics in aquatic culture shows promise in mitigating the negative impacts of pathogen outbreaks. This approach can help reduce economic losses caused by fish mortality and minimize the need for antibiotics to treat bacterial pathogens, thereby making fish farming more ecologically friendly.

Currently, most probiotics studied in fish culture have been previously researched or used in humans or other animals. There is a need to develop new probiotics specifically tailored for fish. A probiotic is defined as "an organism, whether alive, dead, or a component of a microbial cell, that is administered through feed or rearing water and benefits the host by enhancing disease resistance, health, growth performance, feed utilization, stress response, or overall vigor, partly by modulating the host's microbial balance or the microbial balance of the environment" **(Merrifield *et al.*, 2010)**.

This study aimed to investigate the impact of feed and water probiotics on the growth performance, feed utilization, and water quality of the Nile tilapia (*Oreochromis niloticus*) fingerlings.

MATERIALS AND METHODS

This study was conducted at the hatchery of the Faculty of Fish and Fisheries Technology at Aswan University.

1. Diets formation and preparation:

Three isonitrogenous (30% dietary crude protein) and isocaloric (424.76 Kcal/100 g gross energy) diets were formulated from commercial ingredients (fish meal (65%), yellow corn, wheat bran, soybean meal (48%), rice bran, corn gluten (60%), fish oil, vitamins, and mineral mixture). The chemical analysis is presented in Table (1).

The feed was finely ground and sieved before adding probiotics. The probiotics were added at two different levels (0.5 and 1.5g/ kg) and thoroughly mixed to ensure uniform distribution. After that, warm water was added to the mixtures for pelleting.

Experimental treatments were as follows:

Control: (P_0) "No probiotics"

$Pd_{0.5}$: (0.5 g Probiotics / Kg diet)

$Pd_{1.5}$: (1.5 g Probiotics / Kg diet)

$Pw_{0.5}$: (0.5 g Probiotics / Kg diet + 0.05 g probiotics /L water)

$Pw_{1.5}$: (1.5 g Probiotics / Kg diet + 0.05 g probiotics /L water).

Table 1. Chemical analysis of the experimental diets

Chemical analysis	
Moisture	3.53%
Crude protein (CP) (N × 6.25)	30%
Ether Extract (EE)	8.15%
Crude fiber (CF)	5.40%
Ash	7.96%
Carbohydrate (NFE) *	44.56%
Gross energy kcal/100g **	424.76

* %Nitrogen free extract (%NFE) = 100 – (%water + %CP + %CF + %Ash + %EE)

** Gross energy was calculated from their chemical composition using the factors 5.65, 9.45, and 4.0 (Kcal GE/Kg DM) for crude protein, ether extract, and nitrogen free extract, respectively **(Jobling, 1983)**.

2. Experimental aquarium

Rectangular glass aquariums (15 aquariums) with dimensions (length*width*height) (70 x 30, 40cm) were used. The Nile tilapia fingerlings (7g) were transferred from the Sahary hatchery in Aswan to the experimental aquariums at a rate of 10 fingerlings/aquarium. Each aquarium was provided with continuous artificial aeration (an air stone was placed in each aquarium)

3. Water quality parameters

Parameters of water quality were dailymonitored daily. Dissolved oxygen (mg/L) was measured with an oxygen meter (Milwaukee MW600 PRO). The measurement of total ammonia nitrogen (TAN) (mg/L) was conducted using the ammonia medium-range indicator (DUMA) (re0603-A, re0603-B, re0603-C, re0603-D, range 0–8 mg/L, volume 15mL). Other water quality parameters were measured using a water quality meter (3 * 1) with the following ranges: pH from 0–14, temperature from 0–50°C, and total dissolved solids (TDS) from 0–1999ppm.

One-third of the aquarium water was daily changed in the no-probiotic treatments, while the water in the probiotic treatments remained unchanged throughout the experiment.

4. Growth performance and food utilization

Growth performance and food utilization indicators were calculated according to the following equations:

- **Weight Gain (WG) g =** FBW – IBW
- **Specific Growth Rate (SGR) %/day =** (ln FBW-ln IBW) 100 / days

  Since: **IBW**= initial body weight; **FBW**=final body weight
- **Survival rate (SR) %=** 100* (final number of fish/initial number of fish)
- **Condition factor (K) =** (BW*100 (g)/ L^3 (cm)
- **Feed conversion ratio (FCR) =** feed intake/ weight gain
- **Protein efficiency ratio (PER) =** protein gain / protein intake
- **Protein productive value (PPV) % =** 100 * (protein gain/protein intake)

5. Statistical analysis

The data attained were analyzed statistically by one-way ANOVA via the statistical programme SPSS, version 22.0 for Windows. The means ± standard errors were used to express all the data. The collected data performed tests for homogeneity of variance and normality of distribution, and the differences were examined using the least significant difference (LSD) test. The significance level was considered to be 5%.

RESULTS

1. Survival and growth performance

Probiotics are effective alternatives to traditional antibiotics and play a crucial role in activating key vitamins, including cobalamin (B12), biotin (B7), and vitamin K, which can enhance growth performance **(El-Saadony *et al.*, 2021; Ahmadniaye *et al.*, 2023)**. Incorporating probiotic supplements into the diet is a proven nutritional strategy for improving growth performance and behavioral patterns in the Nile tilapia **(Sakr, 2003)**. Due to their exceptional properties and health benefits, probiotics are increasingly promoted and sold as quick water enhancers and feed additives **(Jahangiri & Esteban, 2018)**.

The present study presents the growth performance, condition factor (K), and survival rate of *Oreochromis niloticus*, as shown in Table (2). The control group and all probiotic-treated groups exhibited significantly higher (P < 0.05) final body weights, weight gains, and specific growth rates compared to the Pw0.5 group, while no significant difference was observed between the Pw0.5 and Pw1.5 groups. Additionally, the condition factor showed no significant differences (P> 0.05) among the experimental groups.

These findings are inconsistent with those of several authors **(Wang *et al.*, 2017; Xia *et al.*, 2020; Tabassum *et al.*, 2021; Hendam *et al.*, 2023; Munni *et al.*, 2023; Youssef *et al.*, 2024)**, who reported increased growth, survival, and specific growth rates, along with improved growth performance in the Nile tilapia treated with probiotics compared to control diets. The discrepancies may be attributed to variations in probiotic levels, types of probiotics used, experimental conditions, and the duration of the studies.

Furthermore, no significant differences ($P > 0.05$) were found in the survival rates between the control group and the probiotic-treated groups. This aligns with the findings of several authors **(Shelby *et al.*, 2006; Abd El-Rhman *et al.*, 2009; Apún-Molina *et al.*, 2009; He *et al.*, 2013)**, who noted that fish fed probiotics for eight weeks did not exhibit a better survival rate than the control group. However, these results contrast with those of **El-Saadony *et al.* (2021)**, who found that probiotics increased the survival rate of treated groups compared to the control, particularly in the presence of pathogens.

Table 2. Final body weights (FBW), weight gain (WG), specific growth rates (SGR), condition factor (K) and survival rate of the Nile tilapia (*Oreochromis niloticus*) fed on different levels of probiotic (data expressed as: mean ± standard error)

Treatment	FBW	WG	SGR	K	SR
P_0	22.77±0.43 [a]	15.77±0.43 [a]	1.31±0.02 [a]	1.69±0.03 [a]	100±0.00 [a]
$Pd_{0.5}$	21.89±0.46 [a]	14.89±0.46 [a]	1.26±0.02 [a]	1.69±0.04 [a]	100±0.00 [a]
$Pd_{1.5}$	21.53±0.22 [a]	14.53±0.22 [a]	1.25±0.01 [a]	1.68±0.01 [a]	100±0.00 [a]
$Pw_{0.5}$	19.18±1.13 [b]	12.18±1.13 [b]	1.11±0.07 [b]	1.56±0.13 [a]	100±0.00 [a]
$Pw_{1.5}$	20.83±0.53 [ab]	13.83±0.53 [ab]	1.21±0.03 [ab]	1.77±0.15 [a]	100±0.00 [a]

Values within columns with the same superscript are not significantly different ($P > 0.05$)

2. Feed utilization

The effect of probiotics on feed utilization of the Nile tilapia (*Oreochromis niloticus)* is shown in Table (3). There were no significant differences in the feed conversion ratio (FCR) and protein efficiency ratio (PER) among the experimental groups compared to the Pw0.5 group, although the control group recorded the best results. The protein productivity value was significantly the highest in the control group, while the Pw0.5 group had the lowest significant value, with no statistical difference observed among the Pd0.5, Pd1.5, and Pw1.5 groups.

Table 3. Feed conversion ratio (FCR), protein productive value and protein efficiency ratio (PER) of the Nile tilapia (*Oreochromis niloticus*) fed on different levels of probiotic (data expressed as: mean ± standard error)

Feed utilization parameter	FCR	PPV	PER
P_0	1.36±0.03 [a]	27.75±0.40 [a]	1.63±0.03 [a]
$Pd_{0.5}$	1.43±0.03 [a]	26.09±0.64 [ab]	1.55±0.04 [a]
$Pd_{1.5}$	1.41±0.03 [a]	26.74±0.54 [ab]	1.57±0.03 [a]
$Pw_{0.5}$	1.64±0.10 [b]	23.38±1.51 [b]	1.36±0.08 [b]
$Pw_{1.5}$	1.47±0.09 [ab]	25.89±1.74 [ab]	1.52±0.10 [ab]

Values within columns with the same superscript are not significantly different ($P > 0.05$).

These results contradict the outcomes obtained by **Merrifield *et al*. (2010)**, who found that fish given a combination of two probiotics bacterial strains (*B. subtilis* and *B. licheniformis*) showed notable improvements in their specific growth rate (SGR), feed conversion ratio (FCR), and protein efficiency ratio (PER). Additionally, **El-Haroun *et al*. (2006)** found that feed utilization of the Nile tilapia, including SGR, PER and PPV was higher in the treatments induced with probiotics than in the control diet.

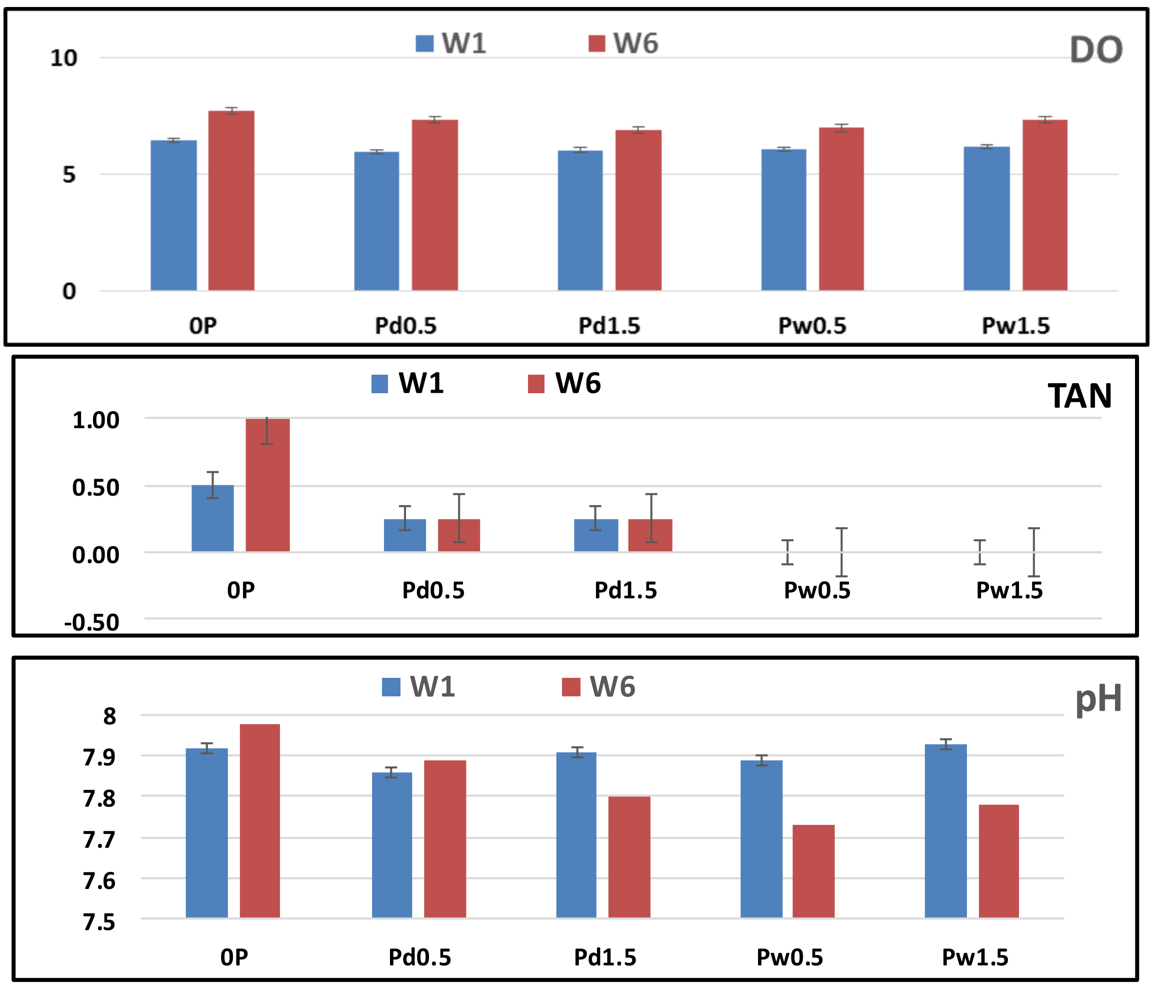
In this study conducted in Aswan under extremely high temperatures, it was observed that not changing the water for groups treated with probiotics to less than 1.5g of water (Pw1.5=1.5g Probiotics/Kg diet+0.05 g probiotics/L water) may indicate that this level is not effective in improving water quality to enhance growth performance. Therefore, increasing the level of water probiotics to 1.5g resulted in improved growth performance indicators.

3. Water quality

The water quality parameters can be found in Fig. (1). The research results showed that all water quality factors met the appropriate limits for tilapia farming, as reported by **(Boyd & Tucker, 2012)**. It was observed that different levels of probiotics had a clear effect on water quality. Probiotic bacteria were found to improve water quality and increase levels of dissolved oxygen (DO). Maintaining proper DO levels is crucial for supporting fish life in water, as mentioned by **Kord *et al*. (2022)**. The DO values of the $Pw_{0.5}$ and $Pw_{1.5}$ treatments after the sixth week were higher compared to the other groups, with no significant differences.

The groups treated with Pw0.5 and Pw1.5 showed the lowest levels of total ammonia (0mg/ l). Ammonia and other wastes of nitrogenous are major elements in intensive fish culture. According to **Naiel *et al*. (2022)**, the movement of these substances is important to prevent fish from dying and accelerate the aquaculture system's supportability. Results demonstrated that water probiotics could reduce the average ammonia levels in the culture water.

The findings of this study align with **Khademzade *et al*. (2020)**, who found that administering two strains of bacteria resulted in a significant reduction in nitrogen concentration in the culture water. Correspondingly, **John *et al.* (2020)** noted that introducing probiotic strains to tilapia's (*O. mossambicus*) rearing water led to a significant decrease in ammonia concentration. *Bacillus* species, as observed by **Rout *et al*. (2017)** and supported by **Su *et al*. (2020)**, possess the ability to eliminate various varieties of nitrogen from host wastewater. This species plays a crucial role in adjusting the nitrogen cycle during processes such as ammonia, nitrification, de-nitrification, and nitrogen fixation. No significant differences among treatments were observed in terms of total dissolved solids (TDS), pH, and temperature.



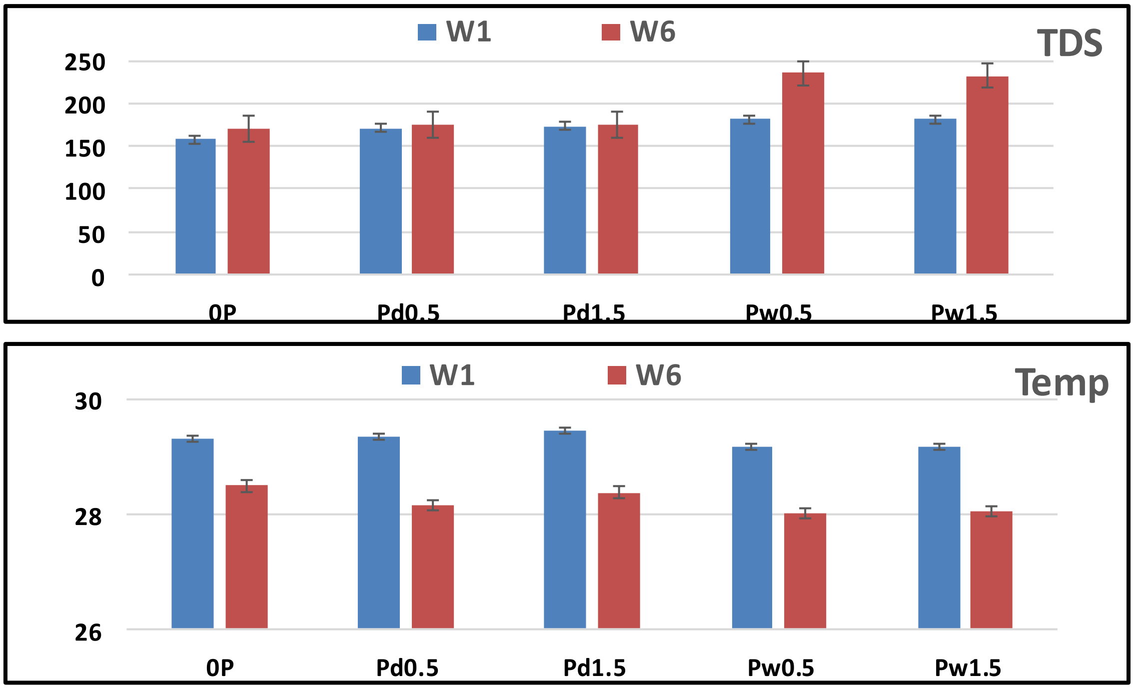


Fig. 1. Effect of probiotics on dissolved oxygen, total ammonia nitrogen, pH, total dissolved solids, and temperature of experimental water of the Nile tilapia (*Oreochromis niloticus*) fingerlings fed on different levels of probiotics

CONCLUSION

This study concluded that using probiotics in feed did not have a significant impact on growth performance and feed utilization compared to the control treatment. However, when aquatic probiotics were used in combination with food probiotics at a minimum of 1.5g probiotics/kg diet + 0.05g probiotics/L water, it effectively improved parameters of water quality, remarkably reducing total ammonia levels.

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