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Commercial Probiotic Usage to Improve Semi-Intensive Tilapia Production System Under Egyptian Conditions

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ABSTRACT

Aquaculture is the fastest-growing sector of animal food production worldwide. However, many challenges face aquaculture farms, with two of the most critical being the high costs of production and the prevalence of diseases, which cause significant mortality, especially during the summer months. This study evaluated the effects of adding a commercial probiotic as a water additive for the Nile tilapia (*Oreochromis niloticus*) raised in semi-intensive earthen pond systems. The experiment was designed as a field study conducted on farms in various governorates. Four farms were selected, each with eight 2.5-acre ponds. Four ponds were treated with probiotics (W/Pro), while the other four served as control ponds without probiotic addition (W/OPro). On each farm, two ponds were used: one for probiotic addition (W/Pro) and the other as a control (W/OPro). Each pond was stocked with 12,000 fingerlings (5 grams each) per acre. The commercial probiotic (Aqua Star®) was added to the experimental ponds at a rate of 200 grams per acre every two weeks for 180 days. At the end of the evaluation, total ammonia nitrogen (TAN), unionized ammonia (NH_3), and nitrite (NO_2) levels were significantly ($P< 0.05$) reduced in the W/Pro ponds compared to the W/OPro ponds. However, total dissolved solids (TDS), pH, and dissolved oxygen (DO) showed no significant differences ($P< 0.05$) between the two groups. Growth parameters such as final weight, weight gain, daily weight gain, specific growth rate (SGR), total length, standard length, and condition factor were significantly ($P< 0.05$) improved in the W/Pro ponds. Pond productivity per acre and survival rates were also significantly ($P< 0.05$) higher in the W/Pro ponds compared to the W/OPro ponds. However, there were no significant differences ($P< 0.05$) in feed conversion ratio (FCR), protein efficiency ratio (PER), or feed efficiency between the two groups. In conclusion, this study demonstrated that the use of probiotics in semi-intensive aquaculture systems for the Nile tilapia offers significant benefits, including improved water quality, growth performance, body measurements, pond productivity, and survival rates.

INTRODUCTION

Fish is one of the most popular trading food and feed commodities on the planet, and it is not only contributing to food security but also has enormous economic value **(Tidwell & Allan, 2012)**. For the last three decades, aquaculture has been the fastest growing animal food production on the planet, with production (excluding aquatic plants) increasing at an average compounded rate of 8.1 percent per year since 1981 compared with 3.0 percent for terrestrial farmed meat production (**FAO, 2008, 2009)**. World aquaculture production reached an all-time high of 114.5 million tons in live weight in 2018, with a farm gate sale value of USD 263.6 billion, 82.1 million tons of aquatic animals (USD 250.1 billion), 32.4 million tons of aquatic algae (USD 13.3 billion), and 26,000 tons of ornamental seashells and pearls made up the total production (USD 179 000) **(FAO, 2020)**. Aquaculture is a vital and rapidly expanding food sector in Egypt. According to the **FAO (2020)**, Egypt ranks as the 6th largest aquaculture producer globally and the largest in Africa. In fact, the aquaculture sector in Egypt produces significantly more fish than the fisheries sector. In 2018, aquaculture accounted for nearly 80.71 percent of total fish production in Egypt, while fisheries contributed only 19.29 percent of total fish production, indicating a significant increase in aquaculture share from 47 percent in 2000 **(GAFRD, 2018)**. In Egypt, aquaculture production is dominated by a few species such as tilapia, carp, and mullet, which account for roughly 93 percent of total production. The Egyptian tilapia production was 1051,444 tons (67.34 percent), mullet was 242,071 tons (15.50 percent), carp was 180,900 tons (11.59 percent), and the gilthead sea bream was 29,994 tons (1.92 percent), and the European sea bass was 24,914 tons (1.60 percent) according to the **GAFRD (2018)**. Many problems stand against aquaculture farms, the most vital ones are the high cost of production requirements, operation cost (including feeds), lack of proper facilities, and disease problems (which cause high mortalities, mostly in summer months). Main challenges facing aquaculture industry in Egypt are water resources, land licensing procedures, aquatic animal diseases, energy and feed quality. Numerous methods have been explored to overcome aquaculture problems, especially diseases; those methods include treatment with antibiotics, disinfectants, immune enhancers, and using higher feed qualities such methods resulted in lowering problems effects but didn’t solve them completely. One of the promising techniques is the treatment by probiotics; a new practice can lead to higher quality and quantity productions by lowering vulnerability and decrease mortalities. The general idea of “probiotic” is the addition of particular balanced amounts of beneficial bacteria such as lactate and bacillus bacteria into farming environment, which in turn competes with pathogenic organisms, enhances growth rates, and provides no harmful effects. Other beneficial effects include improving the digestibility of nutrients, increasing tolerance to stress, and encouraging reproduction (in hatchery processes). Probiotics don’t work only within aquatic animal body, they can also affect the farming environment by improving water quality and controlling pathogens presence, reducing total ammonia nitrogen, improving the pond’s bottom through reducing the accumulation of wastes and reducing the amounts of hydrogen sulfide gases in water. Therefore, in the present study, the Nile tilapia (*Oreochromis niloticus*) raised in semi-intensive earthen ponds system were examined to determine the effects of commercial probiotics used as water addition on water quality, production efficiency, growth performance, feed utilization and body composition.

MATERIALS AND METHODS

1. Experimental design

The experiment was conducted to be a field experiment using farms in different governorates to study the effect of continuous preventive addition of probiotics in ponds to enhance the culture process and help solve its problems. Four farms were selected in four different governorates (Kfr El-sheikh, Port-Said, El-Behera and Ismailia). Each farm has two ponds in which one of them is used with probiotic (W/Pro), and the second one is used as the control pond without probiotic (W/OPro). Thus, the experiment was consisted of eight ponds separated into two groups. Group W/Pro was consisted of four ponds with probiotic, while group W/OPro included four ponds that were used as control ponds.

2. Pond preparation

After the previous season, the pond bottoms were dried to eliminate unwanted fish species. The dikes and fishing ponds were maintained, and the soil inside the ponds was allowed to dry until it cracked. Conditioning was carried out by spreading a layer of lime (100kg per 2.5-acre pond) across the pond bottom for two weeks. Fifteen days after liming, fertilization was applied using mono super phosphate (50kg per 2.5 acres) to promote the growth of fish food organisms.

3. Fingerlings reception

Experimental fingerlings (5g) were received in the same period from a specific hatchery for each farm and were acclimated to be stocked in each pond as 12000 fingerlings/ acre stocking density.

4. Fish feeding

The fish were fed by using feed from a specific commercial feed source (Skretting Egypt) with a protein content of 30% throughout the culture period. The feeding was carried out using a proportion to the live weight that decreased with the increase in the weight of the fish in the pond according to **Eurell *et al*. (1978)** and **Reinitz(1983)**, and it was fixed for all the ponds in the four farms.

5. Probiotic addition

The probiotic used in the experiment, "Aquastar® Pond," is a product of DSM Company. It contains *Bacillus subtilis* spores (DSM 21287) at 5×10^{11} CFU/kg, *Enterococcus faecium* (DSM 3530) at 7.5×10^{11} CFU/kg, and *Pediococcus acidilactici* (DSM 16210) at 7.5×10^{11} CFU/kg. The preventive dose of the probiotic, 200 grams per acre, was applied from the beginning of the culture process, based on the recommendation of the producing company. To prepare the treatment, half a kilogram of the product was mixed with 15 liters of pond water to activate the beneficial bacterial cells. The mixture was continuously stirred for 20 minutes before being sprayed across the pond surface, following the wind direction. This application was repeated every two weeks.

6. Fish sampling

Fish samples were taken at 3 stages (80 days,120 days and at the end of the experiment at 180 days) by using the same standard mesh size net that is able to catch the smallest fish in the pond from a specific point throughout the cultivation period then measurements were taken and recorded in a file for each farm.

7. Measurements

7.1. Growth performance and feed utilization parameters

Growth performance was evaluated using the following parameters:

1. **Specific Growth Rate (SGR % day^{-1})**: SGR = [(ln Wt – ln W0) / t] × 100; where, ln is the natural logarithm; Wt is the final weight; W0 is the initial weight, and t is the rearing duration in days **(Goda *et al.*, 2007)**.

2. **Weight Gain (WG)**: WG = Wt – W0; where, Wt is the final weight, and W0 is the initial weight **(Goda *et al.*, 2007)**.

3. **Length Gain (LG)**: LG = Lt – L0, where Lt is the final length, and L0 is the initial length **(Panase & Mengumphan, 2015)**.

4. **Feed Conversion Ratio (FCR)**: FCR = Feed intake / Weight gain **(Goda *et al.*, 2007)**.

5. **Protein Efficiency Ratio (PER)**: PER = Wet weight gain (g) / Total protein intake (g) **(Ayisi *et al*., 2017)**.

6. **Condition Factor (CF)**: CF = Total weight / (Standard length^3) **(Leitritz & Lewis, 1980)**.

At the end of the experiment, all fish in each pond were netted and counted to determine the survival rate:
Survival rate = (Number of fish at the end of the study / Number of fish initially stocked) × 100 **(Ayisi *et al.*, 2017)**.

Additionally, the total fish weight in each pond was recorded to determine the final production.

7.2. Water quality parameters

Temperature, TDS, pH, DO, TAN and NO_2 measurements were evaluated through the use of specialized devices. Water samples (1Liter/sample) were taken monthly from above the bottom of the ponds by using a sampling bottle. For the measurements of dissolved oxygen, pH and temperature, were measured directly from the pond at a depth of one meter approximately from a specific point throughout the cultivation period by using portable devices, and then measurements were taken and recorded in a file for each farm **(Boyd, 1979; APHA, 1995)**.

8. Devices and equipment

For aeration, 1.5 HP paddle aerators were used in the experimental ponds throughout the day. The same water management practices were applied across all farms. Water quality parameters were monthly measured. Dissolved oxygen (DO) was measured using an ADWA AD630 portable DO meter, while pH and temperature were recorded with an ADWA AD130 portable pH meter. Total dissolved solids (TDS) were measured using an ADWA AD410 portable TDS meter, and total ammonia nitrogen (TAN) was determined with a MILWAUKEE MI405 TAN portable meter. Nitrite levels were measured using a LAMOTTE 3110-01 portable meter. Fish samples were weighed using an SF-400A electronic compact scale, and total and standard lengths were measured with a standard ruler fixed on a 30cm board.

9. Statistical analysis

Data for water quality and growth performance were analyzed using the two-way ANOVAwith interaction. Data for feed conversion rate (FCR), feed efficiency (FE) , protein efficiency rate (PER), production per acre and survival rate % were analyzed using the T-test procedure of the SPSS computer software (SPSS version 25.0 (2017)). Differences between means were assessed using Duncan'smultiple-range test, and effects with a probability of P<0.05 were considered to be significant.

RESULTS

1. Water quality parameters

As shown in Table (1), regarding province, there was no significant difference (P< 0.05) in water temperature (26.18–27.99˚C), pH (8.31–8.38), total ammonia nitrogen (1.02–1.16mg/l), unionized ammonia (0.117–0.182mg/l) and nitrite (0.057–0.065mg/l) mean values. There was a significant difference (P< 0.05) in dissolved oxygen (7.26–5.69mg/l) and total dissolved solids (0.057–0.065mg/l); the highest dissolved oxygen was recorded in port-said (7.26mg/l), while the lowest dissolved oxygen was recorded in El-

Behera (5.69mg/l). The highest dissolved solids value was noticed in Kfr El-sheikh (7.26mg/l), and the lowest in Ismailia (5.69mg/l).

Regarding probiotic treatment, the use of probiotics showed no significant differences (*P*> 0.05) in water temperature (26.96–26.92°C), dissolved oxygen (6.92–6.37mg/l), pH (8.31–8.37), and total dissolved solids (2.33–2.31mg/l). The highest values for temperature, dissolved oxygen, and total dissolved solids were observed in the treated ponds (W/Pro), while the highest pH was recorded in the control ponds (W/OPro). However, the addition of probiotics significantly (*P*< 0.05) reduced total ammonia nitrogen (TAN), unionized ammonia (NH3), and nitrite (NO2) levels in treated ponds, with levels of 0.5, 0.054, and 0.02mg/l, respectively, compared to 1.64, 0.25 and 0.1mg/l in the control ponds (W/OPro).

Table 1. Water quality parameters for the Nile tilapia fingerlings (*O. niloticus*) in different provinces reared for 6 months with and without the usage of commercial probiotic containing *Bacillus subtilis spores, Enterococcus faecium*, and *Pediococcus acidilactic*

Item		Water temperature ◦C	Dissolved oxygen (mg/l)	pH (mg/l)	Total dissolved solids (mg/l)	Total ammonia nitrogen (mg/l)	Ammonia (mg/l)	Nitrite (mg/l)
Province	**Kfr El-sheikh(K)**	27.35	6.45^{ab}	8.38	2.89^{b}	1.05	0.16	0.06
	Port-Said(P)	27.99	7.26^{b}	8.31	2.72^{ab}	1.04	0.18	0.06
	El-Behera(B)	26.18	5.69^{a}	8.31	1.84^{a}	1.16	0.14	0.06
	Ismailia(I)	26.25	7.18^{b}	8.37	1.84^{a}	1.03	0.12	0.06
SE		±1.41	±0.48	±0.08	±0.38	±0.20	±0.04	±0.01
Probiotic	**W/Pro**	26.96	6.92	8.32	2.33	0.5^{a}	0.05^{a}	0.02^{a}
	W/OPro	26.92	6.37	8.37	2.31	1.64^{b}	0.25^{b}	0.1^{b}
SE		±1	±0.34	±0.06	±0.27	±0.14	±0.03	±0.01
Interaction	**K W/Pro**	27.25	6.77	8.36	3	0.53^{a}	0.06^{ab}	0.01^{a}
	K W/OPro	27.45	6.01	8.39	2.78	1.58^{bc}	0.25^{c}	0.1^{b}
	P W/Pro	28.06	7.45	8.19	2.72	0.53^{a}	0.06^{ab}	0.018^{a}
	P W/OPro	27.91	7.08	8.43	2.72	1.54^{bc}	0.3^{c}	0.1^{b}
	B W/Pro	26.25	5.81	8.26	1.85	0.69^{ab}	0.07^{ab}	0.03^{a}
	B W/OPro	26.11	5.56	8.36	1.83	1.63^{c}	0.22^{bc}	0.09^{b}
	I W/Pro	26.3	7.55	8.45	1.76	0.25^{a}	0.02^{a}	0.01^{a}
	I W/OPro	26.21	6.81	8.3	1.91	1.8^{c}	$0.21b^{c}$	0.1^{b}
SE		±1.99	±0.68	±0.11	±0.53	±0.29	±0.05	±0.02

*Results relating to water quality of tilapia cultured ponds including water temperature, dissolved oxygen, pH,total dissolved solids,total ammonia nitrogen, unionized Ammonia, and Nitrite are presented as means, standard error (SE) of observations. Means in the same column with different lower-case letters are significantly different (*P*< 0.05) (Duncan test), n = 3.K= Kfr El-sheikh, P= Port-Said, B= El-Behera and I= Ismailia

Meanwhile, interaction results showed that total ammonia nitrogen (TAN), unionized ammonia (NH_3) and nitrite (NO_2) was significantly (*P*< 0.05) reduced in W/Pro ponds. The highest total ammonia nitrogen (TAN) value was recorded in Ismail's W/OPro pond (1.8mg l^{-1}), while the lowest value was recorded in Ismail's W/Pro pond (0.25mg l^{-1}). The highest unionized ammonia was noticed in Port-Said's W/OPro pond (0.3mg l^{-1}), whereas the lowest valuewas recorded in Ismail's W/Pro pond (0.02mg l^{-1}). On the other hand, the highest nitrite value was recorded in Ismail and Port-Said's W/OPro ponds (0.1mg l^{-1}), and the lowest was detected in Kfr El-sheikh, Port-Said and Ismailia's W/Pro ponds (0.01mg l^{-1}). It can be noticed from Table (1) that there were no significant differences (P< 0.05) observed in water temperature, pH and total dissolved solids with 26.11–28.06°C, 8.19–8.45, and 1.76 –3mg l^{-1}, respectively.

2. Growth performance and body measurements

Table 2. Growth performance parameters for the Nile tilapia fingerlings (*O. niloticus*) in different provinces reared for 6 months with and without the usage of commercial probiotic containing *Bacillus subtilis spores, Enterococcus faecium* & *Pediococcus acidilactic*)

Item		Final weight (gram)	Weight gain (gram)	Daily weight gain	Specific growth rate	Total length	Standard length	Condition factor
Province	KfrEl-sheikh(K)	334.14^{ab}	329.1^{a}	1.83^{a}	2.33^{a}	28.6^{c}	24.89^{b}	1.42^{a}
	Port-Said(P)	347.93^{a}	342.93^{ab}	1.9^{ab}	2.35^{ab}	30^{a}	26.32^{a}	1.28^{b}
	El-Behera(B)	321.47^{b}	316.47^{b}	1.75^{b}	2.31^{b}	29.43^{ab}	26.3^{a}	1.25^{b}
	Ismailia(I)	313.03^{b}	308.03^{b}	1.71^{b}	2.3^{b}	29.23^{bc}	25.67^{a}	1.25^{b}
SE		±9.93	±9.93	±0.05	±0.02	±0.26	±0.22	±0.02
Probiotic	With	346.7^{a}	341.7^{a}	1.89^{a}	2.35^{a}	29.44^{a}	25.71^{a}	1.36^{a}
	Without	311.57^{b}	306.57^{b}	1.7^{b}	2.29^{b}	29.21^{a}	25.75^{a}	1.25^{b}
SE		±6.13	±6.13	±0.03	±0.01	±0.19	±0.16	±0.09
Interaction	K W/Pro	356.03^{a}	3510.3^{a}	1.95^{a}	2.37^{a}	28.79^{bc}	24.96^{bc}	1.49^{a}
	K W/OPro	312.19^{bc}	307.19^{bc}	1.71^{bc}	2.29^{bc}	28.4^{c}	24.81^{c}	1.36^{b}
	P W/Pro	360.25^{a}	355.25^{a}	1.97^{a}	2.37^{a}	29.97^{ab}	26.23^{a}	1.33^{bc}
	P W/OPro	335.61^{ab}c	330.61^{abc}	1.83^{abc}	2.33^{abc}	30.13^{a}	26.41^{a}	1.22^{d}

B W/Pro	341.82^{ab}	336.82^{ab}	1.87^{ab}	2.34^{ab}	29.73^{ab}	26.2^{a}	1.29^{c}
B W/OPro	301.12^{c}	296.12^{c}	1.65^{c}	2.27^{c}	29.13^{abc}	25.43^{abc}	1.21^{d}
I W/Pro	328.69^{abc}	323.69^{abc}	1.79^{abc}	2.32^{abc}	29.18^{abc}	25.91^{ab}	1.31^{c}
I W/OPro	297.36c	292.36^{c}	1.62^{c}	2.26^{c}	29.27^{abc}	25.91^{ab}	1.19^{d}
SE	±8.67	±8.67	±0.05	±0.01	±0.263	±0.23	±0.01

*Results relating to growth performance of tilapia cultured ponds including final weight, weight gain, specific growth rate,total length, standard length and condition factor are presented as means, standard error (SE) of observations. Means in the same column with different lower-case letters are significantly different (P< 0.05) (Duncan test), n = 3.

The growth performance parameters of the Nile tilapia (*O. niloticus*) at the end of the experimental period (180 days) are shown in Table (2). The obtained results related with province revealed that there were significant (P< 0.05) differences observed between the provinces in all parameters. The highest final weight, weight gain, daily weight gain, specific growth rate, total length and standard length were recorded IN Port-said ponds with 347.93, 342.93, and 1.9g/ day, 3.235, 30, and 26.32cm, respectively, while the lowest values of the final weight, weight gain, daily weight gain, specific growth rate and condition factor were recorded in Ismalia ponds with 3013.03, 308.03, and 1.71g/ day, 2.3, 1.25, respectively. The lowest total length and standard length were recorded in Kfr El-sheikh, with 28.6 and 24.89, respectively. Moreover, the highest condition factor was recorded in KfrEl-sheikh. Probiotic treatment resulted in higher values of final weight, weight gain, daily weight gain, specific growth rate and condition factor, with a significant difference at P< 0.05. The highest final weight, weight gain, daily weight gain and condition factor were recorded in the W/Pro ponds with 346.7, 341.7, 1.89g/ day and 1.36, respectively, while the lowest values were recorded in W/OPro ponds with 311.57, 306.57, and 1.7g/ day & 1.25, respectively. Regarding interaction between provinces and probiotic addition, probiotic treatment resulted in higher values of all parameters. Final weight, weight gain, daily weight gain, specific growth rate, total length, standard length, and condition factor showed significant differences between the W/Pro and W/OPro groups. The W/Pro group exhibited higher values for final weight, weight gain, daily weight gain, and condition factor, while the W/OPro group showed the lowest results in these parameters.The highest values of final weight, weight gain, daily weight gain, specific growth rate, total length, standard length and condition factor were 356.03g, 3510.3g, and 1.97g/ day, and 2.37, 30.13cm, 26.41cm and 1.49, respectively, while the lowest recorded values were 297.36g, 292.36g, 1.62g/ day, 2.26, 28.4cm, 24.81cm and 1.19, respectively.

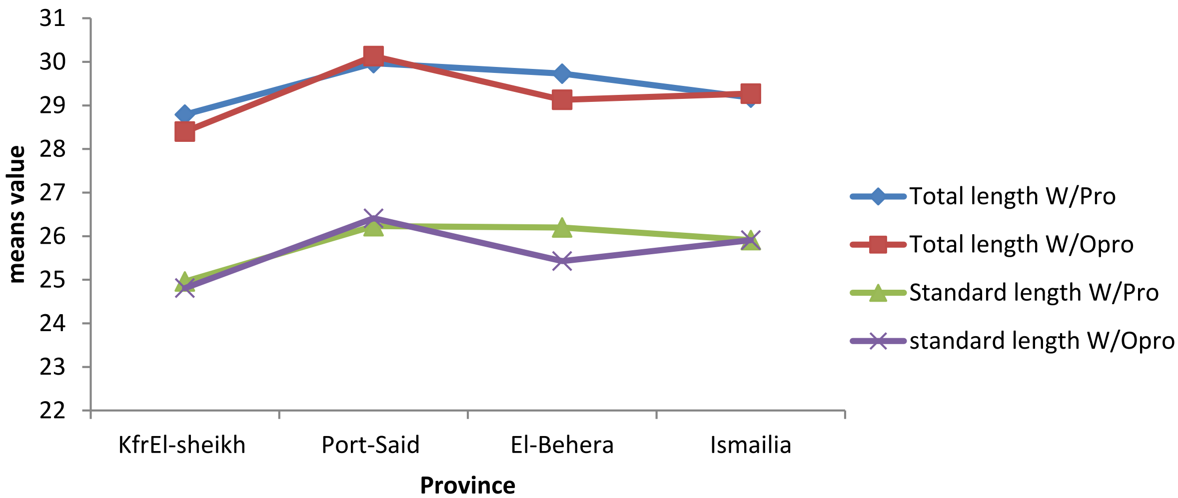

Fig. 1. Fish total length and standard length values in ponds of the Nile tilapia (*O. niloticus*) in different provinces with probiotic (W/Pro) and without probiotic (W/OPro) (*Bacillus subtilis spores*, *Enterococcus faecium and Pediococcus acidilactic*) as water additive for 6 months, n = 3; means ± SE

The total length (cm) was higher in the W/Pro ponds, measuring 29.44cm, while the standard length (cm) was greater in the W/OPro ponds at 25.75cm. The lowest total length was found in the W/OPro group at 29.21cm, and the lowest standard length was in the W/Pro ponds at 25.71cm, with significant differences ($P< 0.05$) between the two groups. Furthermore, interaction results indicated that final weight, weight gain, daily weight gain, specific growth rate, total length, standard length, and condition factor were all significantly improved ($P< 0.05$) in the W/Pro ponds. The highest values were recorded in Kafr El-Sheikh for final weight (356.03g), weight gain (351.03g), and condition factor (1.49), as well as in Port Said for daily weight gain (1.97g/day), total length (30.13cm), and standard length (26.41cm). In contrast, the lowest values were observed in the W/OPro ponds, with Ismailia reporting final weight (297.36g), weight gain (292.36g), daily weight gain (1.62g/day), condition factor (2.26), total length (28.4 cm), and standard length (24.81cm).

3. Production per acre and survival rate (SR%):

The production per acre and survival rate % of the Nile tilapia (*O. niloticus*) at the end of the experimental period (180 days) are shown in Figs.(2, 3).

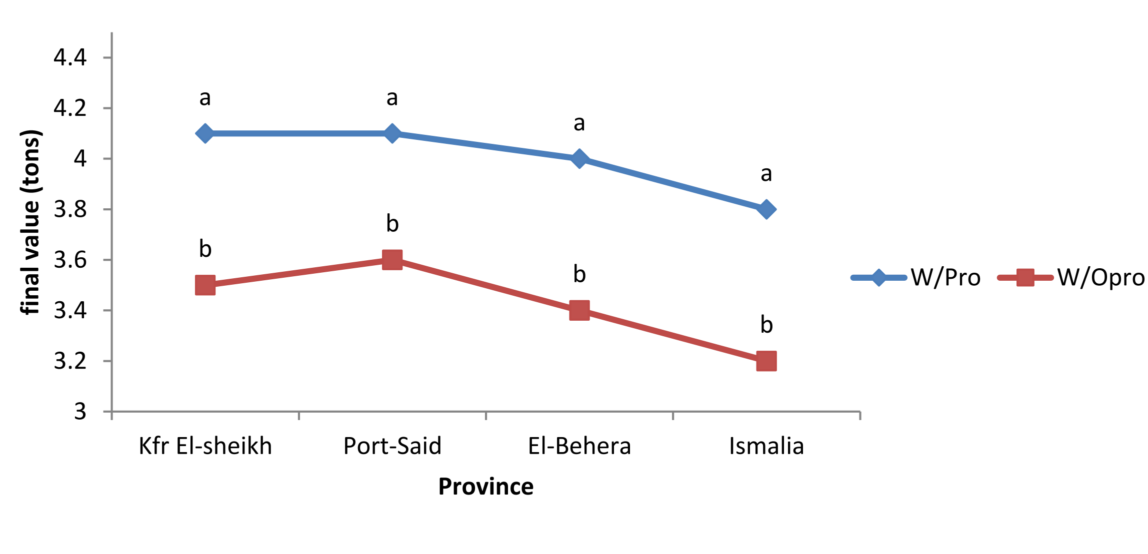

Fig. 2. Production (tons per acre) for the Nile tilapia fingerlings (*O. niloticus*) in different provinces reared for 6 months with probiotic (W/Pro) and without probiotic (W/OPro). The commercial probiotic used contains *Bacillus subtilis spores*, *Enterococcus faecium*, and *Pediococcus acidilactic*. n = 3; means ± SE

The results show that the production per acre was higher in all W/Pro ponds than W/OPro group with a significant difference between them ($P< 0.05$).

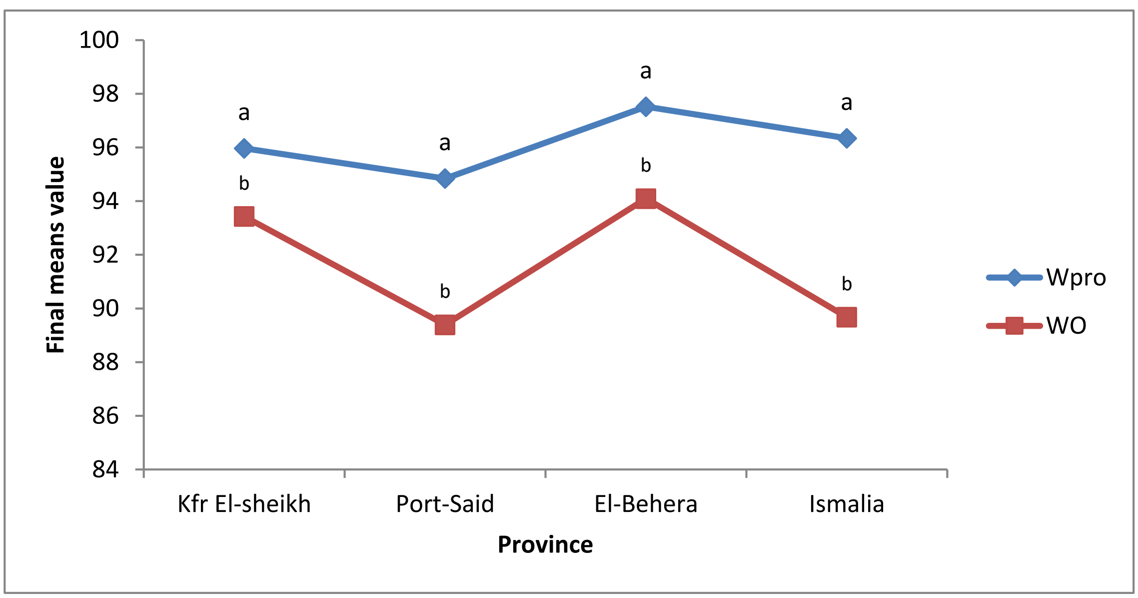

Fig. 3. Survival rate (%) of the Nile tilapia (*O. niloticus*) in different provinces reared for 6 months with (W/Pro) and without (W/OPro) the usage of commercial probiotic. (*Bacillus subtilis spores*, *Enterococcus faecium* and *Pediococcus acidilactic)*. n = 3; means ± SE

Meanwhile, the obtained values revealed that the survival rate (SR%) was significantly ($P< 0.05$) different between W/Pro and W/OPro groups. The highest survival rate was recorded in W/Pro ponds, while the lowest survival rate was recorded in W/OPro ponds.

4. Feed utilization parameters

The feed utilization parameters of the Nile tilapia (*O. niloticus*) at the end of the experimental period (180 days) are shown in Fig. (4).

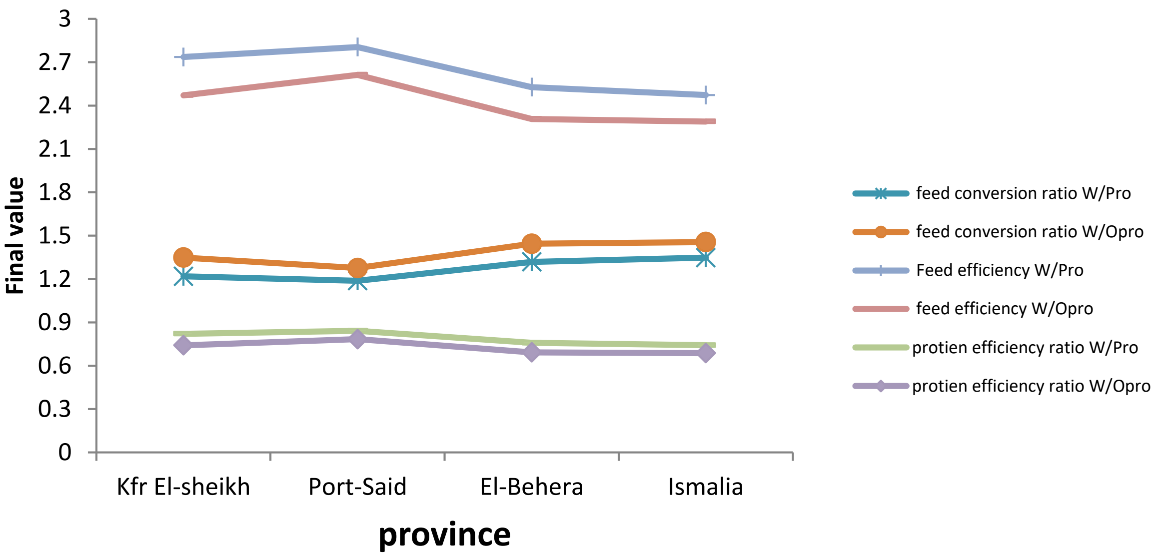

Fig. 4. Feed utilization parameters for the Nile tilapia (*O. niloticus*) in different provinces reared for 6 months with (W/Pro) and without (W/OPro) the usage of commercial probiotic. (*Bacillus subtilis spores*, *Enterococcus faecium*, and *Pediococcus acidilactic)*. n = 3; means ± SE

The results obtained revealed that fish reared in W/Pro group showed an improved feed utilization performance as feed conversion rate (FCR), feed efficiency (FE) and protein efficiency rate (PER) compared to W/OPro group with no significant ($P< 0.05$) differences among them. The highest feed conversion rate (FCR), feed efficiency (FE) and protein efficiency rate (PER) values were 1.26, 2.63 & 0.79, respectively, in W/Pro ponds. Meanwhile, W/OPro group showed the lowest feed conversion rate (FCR), feed efficiency (FE) and protein efficiency rate (PER) values with 1.38, 2.42, and 0.72, respectively.

DISCUSSION

1. Water quality

The majority of studies examining the impact of probiotics in aquaculture so far have employed dietary supplements. However, the possible health benefits of administering probiotics in water have received little attention **(Jahangiri & Esteban, 2018)**. Physical-chemical characteristics of pond water are crucial indicators of its quality and a culture system's capacity to sustain fish production. In particular, dissolved oxygen (DO) is a highly important water quality metric that sustains all life including fish **(Yang *et al*., 2023)**. Several probiotic bacteria species used as water additions in the current investigation had no significant effect on DO levels. This is due to the artificial aeration that all ponds received. The study's stated water quality parameter values fell within the range that is ideal for tilapia production **(Boyd & Tucker, 2012)**. During the experimental period we noticed no significant differences ($P< 0.05$) of probiotic addition on water temperature ranging between 26.11 and 28.06°C, DO 5.56 –7.55mg l^{-1}, pH 8.19–8.45 and TDS 1.76 –3mg l^{-1}.Our results concurred with those of previous studies who found no relationship between probiotic additions and water quality. In this regard, **Wang *et al*. (2008b)** tested water quality in a tilapia culture using a probiotic *Enterococcus faecium* 1×10^7 cfu ml^{-1} by changing 50% of the water every week before the probiotic was added, while **Zhou *et al.* (2010)** tested *Bacillus coagulans* B16 and *Rhodopseudomonas palustris* G06 at a concentration of 1×10^7cfu ml^{-1} as additives in water-reared tilapia, with 50% of the water being changed every two days right before the addition of probiotics. According to these findings, changing the water every two or seven days prevented the accumulation of organic waste and preserved the water's purity. Understanding the dynamics of the nitrogenous compounds in fish culture water, such as total ammonia nitrogen (TAN), unionized ammonia (NH_3) and nitrite (NO_2), is essential for the system's effectiveness. In the current study, adding probiotics to pond water significantly decreased TAN (0.255–0.527mg l^{-1}), NH_3 (0.02–0.066mg l^{-1}) and NO_2 (0.011–0.03mg l^{-1}) levels as compared to the un-treated ponds (1.545–1.8mg l^{-1}, 0.21–0.3mg l^{-1}, and 0.095–0.1mg l^{-1}, respectively). The increased microbial activity that ingested the nitrogenous substances and used them in their metabolism may be responsible for these outcomes. The present study's decreases of nitrogenous compounds are consistent with earlier findings. In this regard, **El-Kady *et al*. (2021)** elucidated that, in comparison with the control treatment, commercial probiotics used as water additions decreased the NH_3 and total ammonia-nitrogen. **Song *et al*. (2011)** postulated that levels of sulphide metabolites, total ammonia, and nitrite were significantly decreased after probiotic addition.**Wang *et al*. (2007)** mentioned that phosphorus and ammonia levels in shrimp water ponds can be reduced by adding probiotics of *Bacillus* sp. to pond water. **John *et al*. (2020)** used *Pseudomonas stutzeri*, *Bacillus cereus*, and *Bacillus amyloliquefaciens* as a mixture of microbial product in tanks of *Oreochromis mossambicus* fingerlings. Researchers observed that this combination of bacteria effectively reduced nitrogenous particles in tanks. Additionally, probiotics may have increased the amount of nitrifying bacteria in ponds' water. According to **Wang *et al*. (2005)**, probiotics dramatically increased the amount of nitrifying bacteria *Nitrosomonas* and *Nitrobacter* in shrimp ponds.

2. Growth performance measurements

Since probiotics were first employed in aquaculture, an increasing number of studies have shown that they can improve the health and development of aqua cultured animals **(Lara-Flores *et al*., 2003; Carnevali *et al*., 2004; Macey & Coyne, 2005; Wang *et al*., 2005; Balcázar *et al*., 2006; Gatesoupe, 2007; Wang, 2007)**. In our study, ponds supplied with probiotic-treated water demonstrated significant improvements in growth performance parameters, including final weight, weight gain, daily weight gain, specific growth rate, total length, standard length, and condition factor, with all parameters showing significant differences ($P< 0.05$) within the same province. These findings align with those reported by **Wang *et al.* (2008b)**, who observed similar results in tilapia (*Oreochromis niloticus*) treated with the probiotic *Enterococcus faecium* at a concentration of 1×10^7 CFU/ml in aquarium water over a 40-day period. The probiotics had a better final weight and daily weight increase compared to the control group, but they had no impact on the survival rate and length gain. Moreover, **Zhou *et al*. (2010)** examined additives in water-reared with tilapia (*O. niloticus*) for 40 days using *Bacillus coagulans* B16 and *Rhodop seudomonas palustris* G06 at a concentration of 1×10^7cfu ml^{-1}. In comparison with fish treated with *Bacillus subtilis* B10 and the control group, their findings revealed significant increases in final weight, daily weight gain, and specific growth rate ($P< 0.05$); however, there was no difference was detected in the survival rate. This is consistent with the results reported by **Sutthi *et al.* (2018)**, who found that the growth performance of the Nile tilapia fed in probiotic-treated water with *Saccharomyces cerevisiae* and *Bacillus* spp. was significantly enhanced ($P< 0.05$) in terms of final body weight, weight gain, and average daily growth. However, no significant differences were observed in length gain values compared to the control group. **Aly *et al*. (2008)** were examined *Bacillus pumilus* for its possible probiotic benefits in tilapia (*O. niloticus*) culture, and it was shown that a small amount ($106g^{-1}$ meal supplied) of the bacteria significantly increased weight gain after two months of usage. Similar results were noticed by **Taoka *et al*. (2006)**, who reported that commercial probiotics, which contain *Bacillus subtilis*, *Lactobacillus acidophilus*,*Clostridium butyricum* and *Saccharomyces cerevisiae*, affected the growth of the Japanese flounder (*Paralichthys olivaceus*) and found that adding probiotics to the diet or growing water promoted the flounder's growth. In this study, it was evident that giving tilapia, *O.niloticus, Bacillus subtilis spores* (DSM 21287), *Enterococcus faecium* (DSM 3530 and *Pediococcus acidilactic* (DSM 16210), probiotics through tank water, had a positive impact on their growth performance. Similarly, **El-Okaby (2015)** discovered that fingerlings of *Sparus aurata* had improved growth performance, feedutilization, condition factors, and survival percentage when a commercial probiotic was introduced to the rearing water.

3. Feed utilization parameters

The performance and welfare of farmed animals are directly affected by the quality of the aquatic environment **(Hura *et al*., 2018)**. Many studies support the beneficial benefits of probiotic therapy in tilapia *O. niloticus*,**(Wang *et al*., 2008a; Zhou *et al*., 2010; Wang *et al*., 2017; Madani *et al*., 2018; Sutthi *et al*., 2018)**, the Pacific white shrimp *Penaeus vannamei* **(Xia & Zhu, 2014)**, the gilthead sea bream *Sparus aurata* **(Lotfy, 2015)**, and the European sea bass *Dicentrachus labrax* **(Aly *et al*., 2016)**. However, the results obtained at the end of the experiment showed that adding probiotics in the water used for fish culture improved tilapia feed utilization parameters compared to the control group. Same results were reported by **Kord *et al*. (2021)**, who mentioned that probiotic species were directly introducedto tilapia *O. niloticus* culture systems to encourage feed intake and nutrient absorption, but feed utilization parameters did not show a significant differences compared to control measurements. **Sutthi *et al*. (2018)** mentioned that adding probiotic to cultured water did not significantlyaffectthe feed conversion ratio of the Nile tilapia (*O. niloticus*).

4. Production per acre and survival rate (SR %)

In fish culture water, microorganisms are essential for controlling production, nutrient cycling, disease outbreaks **(Moriarty, 1997)** and environmental protection. According to the current study, the usage of probiotic significantly increased the pond's productivity per acre ($P< 0.05$) with 4 tons/acre compared to the untreated ponds with 3.42 tons/acre. In agreement with **Kord *et al*. (2021)**, authors suggested that adding probiotics to pond water may enhance fish productivity and improve their health. Similar studies **(El-Haroun *et al*., 2006; Taoka *et al*., 2007; Avella *et al*., 2010; Mandiki *et al*., 2011; Lukkana *et al*., 2015; Silva *et al*., 2015; Adeoye *et al*., 2016; Liu *et al*., 2017)** have shown that *Bacillus* could colonize fish guts and improve organic acid production, digestive enzyme activation, and the detoxification of harmful feed ingredients. This would help sustain good gut health and enhance nutrient digestibility and absorption. Previous studies also noticed that the Nile tilapia growth and productivity were significantly enhanced when raised in culture water containing several probiotic species **(Elsabagh *et al*., 2018a, b; Kord *et al*., 2021)**. In this study, probiotic treatment resulted in significant ($P< 0.05$) higher values of survival rate % in the probiotic-treated ponds. According to **Austin *et al*. (1995)**, **Heyman and Menard(2002)**, **Isolauri *et al*. (2002)** and **Taoka *et al*. (2006)**, there is growing evidence that probiotic bacteria can successfully enhance host innate and adaptive immune responses in addition to other ways that they exert their therapeutic effects. Further, it is believed that the interactions between fish and surrounding microbes have a significant impact on the health and disease of fish **(Ghanbari *et al*., 2015)**. **Zhou *et al*. (2010)** revealed that probiotics added to water have been associated to better aquatic animal growth, survival, and immunological response. Our findings differed from **Chen and Chen (2001)**, who mentioned that fish cultured in water containing *S. cerevisiae* showed greater death rates than fish raised in water containing *Bacillus* spp. or the control group.

CONCLUSION

To put it briefly, the findings of this study about the effects of applying probiotic as water additive in the tilapia controlled semi-intensive earthen ponds culture system was beneficial to the fish since the water's quality, fish growth, survival rate and productivity were all positively enhanced.

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