Desalination and Water Treatment 319 (2024) 100480

Contents lists available at ScienceDirect

Desalination and Water Treatment

journal homepage: www.sciencedirect.com/journal/desalination-and-water-treatment/

Effects of water additive mixed probiotics on water quality, growth performance, feed utilization, biochemical analyses and disease resistance against *Aeromonas sobria* of Nile tilapia

Alya Redhwan[a], El-Sayed Hemdan Eissa[b,*,1], Omaima H. Ezzo[c], Asmaa Sayed Abdelgeliel[d], Mohammad Bodrul Munir[e,*,2], Ahmed Jalal Khan Chowdhury[e,2], Zulhisyam Abdul Kari[f], Muhammad Nur Syafaat[g], Ade Erma Suryani[h], Moaheda E.H. Eissa[i,3], Omayma M. Abd Al-Kareem[j]

[a] *Department of Health, College of Health and Rehabilitation Sciences, Princess Nourah bint Abdulrahman University, P.O.Box 84428, Riyadh 11671, Saudi Arabia*
[b] *Fish Research Centre, Faculty of Agricultural Environmental Sciences, Arish University, El-Arish, Egypt*
[c] *Department of Animal Reproduction and Artificial Insemination, National Research Centre, Dokki, Giza, Egypt*
[d] *Department of Botany & Microbiology, Faculty of Science, South Valley University, Qena 83523, Egypt*
[e] *Faculty of Agriculture, Sinaut Campus, Universiti Islam Sultan Sharif Ali, Brunei Darussalam*
[f] *Department of Agricultural Sciences, Faculty of Agro-Based Industry, Universiti Malaysia Kelantan, Jeli Campus, Jeli 17600, Kelantan, Malaysia*
[g] *National Research and Innovation Agency (BRIN), Jakarta, Indonesia*
[h] *Research Center for Food Technology and Processing, Nasional Research and Innovation Agency, Indonesia*
[i] *Department of Biotechnology, Fish Farming and Technology Institute, Suez Canal University, Ismailia, Egypt*
[j] *Department of Fish Health and Diseases, Faculty of Fish & Fisheries Technology, Aswan University, Egypt*

A B S T R A C T

The present study examined the effects of water additive mixed probiotics on water quality, growth performance, feed utilization, biochemical analyses and disease resistance against *Aeromonas sobria* of Nile tilapia (*Oreochromis niloticus)*. Triplicate groups of Nile tilapia fingerlings (46 g) were fed a commercial diet (ALLER AQUA FEED) in four water treatments control (0.0 g/m3), T1 (0.0010 gm m-3 day-1), T2 (0.0015 gm m-3 day-1), T3 (0.0020 gm m-3 day-1) of probiotic (AquaStar® Pond) for 60 days. The water quality parameters particularly total ammonia nitrogen (TAN) and ammonia (NH3) decreased significantly ($P \leq 0.05$) in all three water treatments when compared to the control treatment. The better values of growth performance, feed utilization and hematology recorded of probiotic groups. The results also showed that fish reared in three water treatments with additive probiotics had a lower mortality when challenged with *Aeromonas sobria*. Hence, the water additive probiotic used in this study is a promising solution as an alternative product to improve the water quality, growth, and health of *O. niloticus*.

1. Introduction

Globally, aquaculture has emerged as one of the fastest-growing agricommodities, accounting for around 50 % of the food fish basket [1].

Tilapia is the second most commercially farmed fish in the world, after carp [2]. In 2018, Tilapia production was approximately 6.882 million tonnes [3], with a projected increase to 7.3 million tonnes by 2030 [4]. The previous ten years have seen a fourfold growth in tilapia output because of the fish's adaptability for aquaculture, consumer acceptance, and steady market prices [5,6].

However, because of the intense cultural methods used in the aquaculture industry, fish raised for aquaculture are susceptible to a wide range of pathogenic organisms. Furthermore, the overuse of chemotherapy has resulted in the development of antimicrobial resistance and irreversible damage to the aquatic ecosystem due to xenobiotic contamination[7]. Diseases and bad environmental conditions continually hinder productivity, resulting in serious financial losses for farmers [8].

The fish farming sector often uses antibiotics to combat disease outbreaks, but because bacteria can become resistant to antibiotics, their effectiveness is not always assured. It affects aquaculture, fish

* Corresponding authors.
E-mail addresses: AMRedhwan@pnu.edu.sa (A. Redhwan), Sayedhemd@gmail.com (E.-S.H. Eissa), hsjewel730@yahoo.com (M.B. Munir), moahedaelsayed@gmail.com (M.E.H. Eissa), OabdelKareem@yahoo.com (O.M. Abd Al-Kareem).
[1] https://orcid.org/0000–0002-7229–2175
[2] https://orcid.org/0000–0003-1162–1702
[3] https://orcid.org/0000-0002-1733-6892

https://doi.org/10.1016/j.dwt.2024.100480
Received 28 August 2023; Accepted 24 March 2024

consumers, and the environment in addition to aquaculture ecosystems [9–11].

Probiotics are single or combined cultures of microbiological communities that, in adequate amounts, may aid in the host's growth and well-being [12–14]. Probiotics have been marketed and sold as an instant water supplement and feed additive because of their special qualities and health benefits [15]. Although probiotics have a wealth of evidence supporting their efficacy and sustainability as a biocontrol strategy, the need for novel probiotic candidates and their scientific validation remains a requirement for the well-being of aquaculture [16].

Probiotics serve numerous functions, including boosting water quality and growth performance, promoting digestion and absorption by improving the microbial balance of the intestine, and so on [15,17].

Direct application of probiotics to water is an excellent way for improving water quality [15]. Probiotics improved water quality parameters like dissolved oxygen, hardness, pH, temperature, and osmotic pressure [18]. Therefore, adding probiotics to fish raising water is a promising way to improve growth, feed efficiency, and disease outbreak management while achieving aquaculture sustainability [19,20]. Additionally, the flavor of the probiotics should preferably be agreeable and appropriate for the species being grown.[11,21,22]. Probiotic supplements are safe for the environment, useful for aquatic species, and do not negatively impact the naturally occurring good bacteria in the surrounding water [8,23,24]. These probiotics may also strengthen the immune system, boost growth and immunity-related genes, boost economic return, and enhance the quality of the water [25]. In contrast, antibiotics are known to negatively impact live biota, particularly in the naturally occurring beneficial bacterial flora, meaning that using them has unfavorable effects and should be avoided [26].

One of the primary causes of infections affecting tilapia is bacterial infection, such as Aeromonas spp., which can result in significant financial losses [27]. Aeromonas spp. are gram-negative, motile, small rods that live in the fish gut and aquatic environment and can induce sickness and death within one week of infection [28]. Skin sores, fin erosions, eye infection, and hemorrhagic septicemia are symptoms of diseased fish [29]. According to Moustafa, Dawood, Assar, Omar, Elbialy, Farrag, Shukry and Zayed [30], Aeromonas infection is the most important impediment to the global expansion of the Nile tilapia business.

Aeromonas sobria is a common water-dwelling bacterium that can infect tilapia either acutely or chronically through its virulence components, which include hemolysin, enterotoxins, and others [31]. Hemorrhagic septicemia caused by *A. sobria* can occur year-round, although it is more common in situations with low water quality, little water change, dense stocking, overwintering, and fluctuating temperatures [32]. According to study by Li and Cai [27], *A. sobria* can cause juvenile tilapia to develop tail rot disease.

Consequently, this study investigated the impact of water additive Mixed Probiotics on water quality, growth performance, feed utilization, biochemical analyses and disease resistance against *Aeromonas sobria* of Nile tilapia (*Oreochromis niloticus*).

2. Materials and methods

2.1. Experimental design

The experimental trial was conducted at a private fish farm, with prior approval of the Faculty of Science, South Valley University Research Ethics Committee (REC-FSCI-SVU-0004/05/24). 240 *Oreochromis niloticus* fingerlings (46 g) were reared in 12 concrete ponds (1 × 1 × 1.2 m, 1 m^3), where 20 fish were stocked per each pond.

Fish were acclimated for 15 days. Probiotic used in this study is AquaStar® Pond is a blend of probiotic bacteria (*Bacillus sp., Pediococcus Sp., Enterococcus Sp.*) and organic carrier. Number of bacteria is min 2 × 10^{12} CFU/kg product (Producer: BIOMIN GmbH. Industriestrasse 21, A-3130, Herzogenburg, AUSTRIA).

Fish were fed on the extruded diets containing 30 % protein ALLER AQUA FEED (https://www.aller-aqua.com/). The proximate chemical composition of the used commercial diet is crude protein (30 %), crude fat (5.2 %), NFE (47.2 %), ash (5.8 %), fibre (4.8 %), gross energy (16.74 MJ), and digestabile energy (8.7 MJ).

2.2. Application protocol

Ponds were assigned to four treatments (triplicate group for each treatment) for 60 days with treatments as follows:

T0 = 0.0 g/m^3.

T1 = 0.0010 gm m^{-3} day^{-1}.

T2 = 0.0015 gm m^{-3} day^{-1}.

T3 = 0.0020 gm m^{-3} day^{-1}.

Fish were fed three times a day at 08:00, 11:00 and 14:00 at 2.5 % of fish biomass. The fish of each experimental group were live-weighed to recalculate the amount of feed consumed during the trial period every two weeks. Water parameters were examined daily during the trial and the treatments were checked under static aerated water conditions with a 30 % water change for the control group and 5 % for all treated group.

2.3. Water quality analysis

Water samples were collected twice a month. Temperature and dissolved oxygen (D.O.) levels were measured with an oxygen meter outfitted with oxygen and temperature sensors. The pH values were recorded using a pH meter. The water's salinity was determined using a refractometer (Erma, Japan), and the unionized ammonia (NH3) was computed using [33]. An ammonia test kit (Advance Pharma, Thailand) was used to assess the pond water's total ammonia nitrogen (TAN).

2.4. Evaluation of the growth parameters and feed utilization

The growth performance, feed utilization, and survival rate were assessed according to average final weight gain, specific growth rate (SGR), and feed conversion ratio (FCR), based on the following equations:

weight gain (WG) = Final weight (g)-Initial weight (g)

Weight gain % (WG%) = (Final weight - Initial weight)/Initial weight × 100·

Average daily gain (g/fish/day): ADG = Wt - W0/n

Where: W0: the initial mean weight of fish in grams.

Wt: the final mean weight of fish in grams.

Where: n: duration period.

Feed Intake (g/fish): The amount of feed given or supplied during the experimental period /fish (g).

Feed conversion ratio (FCR) = Feed intake (g)/Weight gain (g)·

Specific growth rate (SGR) = 100 × (lnW2-lnW1)/T·

Where ln is the natural logarithm, W1 is the initial weight, W2 is the final weight (g), and T is the number of days in the feeding period.

Survival rate (SR) = (Z/X) × 100, Where, Z is the surviving fish number and X is the initial fish number.

2.5. Proximate chemical analysis

Dry matter, crude protein (N 6.25, using Kjeldahl method), ether extract (Chloroform/methanol extraction by Soxhlet extractor), and ash contents of feed ingredients. Fish samples were evaluated after being weighted and dried for 6 h in an oven at 105 C for feed and 70 C for fish [34,35].

2.6. Biochemical analyses

Blood samples were obtained from the caudal vein of fish (three for each replicate) in clean, dry centrifuge tubes, allowed to clot for 15 min, spun at 3000 rpm for 10 min, and then frozen at − 20 °C for biochemical analysis. Henry et al. (1974) employed a colorimetric approach to measure serum total protein (g/dL) and albumin (g/dL). creatinine and urea levels were determined according to Heinegård and Tiderström [36]. Sunderman Jr and Johnson [37] determined serum globulin (g/dL) levels by measuring the discrepancies between total protein (g/dL) and albumin (g/dL). The activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase [26] were determined using the method of Reitman and Frankel [38].

2.7. Experimental challenge

A pathogenic strain of *A. sobria* was used. The resistance of Nile tilapia to pathogenic *A. sobria* was studied after a 60-day experiment in which the fish were cultured in an incubator on nutritional broth for 24 h at 30 °C. Bacterial pellets were produced after 30 min of centrifugation at 3000 g. As mentioned by Schäperclaus, Kulow and Schreckenbach [39], the pellets were suspended in 1.0 mL of 0.1 % peptone water and inoculated with a 0.1 mL dosage of 24 h broth from *A. sobria* (5 105 CFU/mL). In the fish, 1.5 107 cells/mL cell suspension was administered intraperitoneally [40]. The challenged fish were observed daily for 15 days to record mortalities and/or clinical symptoms.

2.8. Statistical analysis

The results for each measured parameter were expressed as means ± standard deviations. Statistical evaluation of results was carried out using the one-way analysis of variance [41], to detect the significance of differences of various parameters among the treatments according to SPSS (version 26).

3. Results

3.1. Water quality

Marked variation in water temperatures and pH levels was detected between the control and supplemented groups. Salinity values were increased in probiotic groups compared to the control group. DO, TAN and Un-ionized ammonia levels were decreased in treatment groups compared to the control group ($p < 0.05$) (Table 1).

3.2. Growth performance

The growth parameters, feed utilization, and survival rate values are presented in Table 2. The data indicated that Nile tilapia reared in water with probiotics (T1, T2, and T3) had higher final body weight, weight gain, weight gain rate, feed intake, biomass and specific growth rate (SGR) than fish fed the control group. The better FCR and survival rate were found in T2 (1.20 % and 98.33 %, respectively).

3.3. Chemical composition

The whole-body proximate compositions (dry matter, protein, and ash content) of Nile tilapia were higher in all probiotic-supplemented groups compared to the control group. In contrast, Ether extract content was decreased in the probiotic groups compared to the control group (Table 3).

3.4. Biochemical markers

Total protein, globulin, and albumin were significantly ($p < 0.05$) higher in the serum of probiotic groups than in the control group. Serum AST, ALT, ALP and urea activities were significantly decreased in supplemented groups. No marked change was observed in creatinine levels (Table 4).

3.5. A. sobria challenge test

As shown in (Fig. 1), adding probiotic to water significantly enhanced ($P > 0.05$) Nile tilapia resistance to *A. sobria* infection compared to the control group. After the fish died, exophthalmia, ascites, tail and fin rots, scalelessness with external hemorrhage, and septicaemic lesions of the internal organs were discovered. Exophthalmia and internal organ septicaemia were clinical signs of mortality.

4. Discussion

The purpose of this study was to assess the growth promoting and immunostimulant effects of probiotic addition to water in Nile tilapia rearing systems, as well as the susceptibility of fish to *Aeromonas sobria* infection. The effects of this probiotic were studied using various factors such as water quality, growth performance, blood biochemical indicators, and histology of the liver and intestine. Aquaculture productivity is strongly reliant on proper water quality [11].

Many research found that using probiotics in aquaculture improved growth performance, survival rate, feed digestion, and animal immunity [43,44].

Water quality parameters may have an impact on the efficacy of probiotic supplementation in aquaculture production [15,45]. Results of our study indicate a clear improvement in water quality, represented by a decrease in total and toxic ammonia levels in probiotics-treated groups. As well, these results are consistent with many previous studies [8,11,13,17,46]. Consequently, these data contributed to the beneficial role of probiotic to enhance the water quality and fish health.

The addition of probiotics to tilapia water enhanced body weight gain, SGR, feed intake, FCR, ADG, biomass, and survival rate when compared to water without probiotics. It has been observed that adding probiotics to fish culture water improved tilapia growth and feed efficiency. Early researchs also demonstrated that probiotic-enriched tilapia rearing water significantly increased growth and production [11,13,47,48].

According to the findings, using probiotic bacteria improved the protein content of fish [8,11,49]. This could be related to increased feed intake, enhanced digestibility and nutrient utilization, and differences in muscle deposition rate [46]. Furthermore, the higher body protein

Table 1
Effect of probiotic use on some water parameters of rearing water of Nile Tilapia (*Oreochromis niloticus*).

Parameters	Control	T1	T2	T3
Temp.[42]	27.270 ± 0.11930^{b}	27.153 ± 0.03^{b}	27.570 ± 0.07^{a}	27.163 ± 0.08^{b}
Salinity (ppm)	214.000 ± 1.52753^{b}	516.333 ± 2.03^{a}	519.333 ± 1.76^{a}	519.667 ± 3.18^{a}
DO_2 (ppm)	7.820 ± 0.05292^{a}	7.793 ± 0.08^{a}	7.800 ± 0.06^{a}	7.803 ± 0.04^{a}
pH	8.167 ± 0.00333^{a}	8.160 ± 0.00^{a}	8.173 ± 0.00^{a}	8.123 ± 0.01^{b}
TAN (ppm)	1.029 ± 0.03438^{a}	0.864 ± 0.02^{b}	0.688 ± 0.00^{c}	0.532 ± 0.00^{d}
NH_3 (ppm)	0.104 ± 0.00153^{a}	0.085 ± 0.00^{b}	0.065 ± 0.00^{c}	0.042 ± 0.00^{d}

Table 2
Effects of probiotic as water additive on the growth performance, fish biomass, and feed utilization of Nile tilapia (O. niloticus).

Parameters	Control	T1	T2	T3
initial fish weight(g)	46.97 ± 0.09^{a}	47.03 ± 0.12^{a}	47.03 ± 0.09^{a}	47.10 ± 0.21^{a}
final fish weight (g)	77.17 ± 0.89^{c}	83.37 ± 1.30^{b}	87.87 ± 1.58^{a}	86.77 ± 0.90^{ab}
Weight gain	30.20 ± 0.80^{c}	36.33 ± 1.18^{b}	40.83 ± 1.51^{a}	39.67 ± 0.86^{ab}
Weight gain %	64.29 ± 1.59^{c}	77.24 ± 2.33^{b}	86.81 ± 3.07^{a}	84.22 ± 1.86^{ab}
Feed intake	48.85 ± 0.09^{a}	48.91 ± 0.12^{a}	48.91 ± 0.09^{a}	48.98 ± 0.22^{a}
Feed conversion ratio (FCR)	1.62 ± 0.04^{a}	1.35 ± 0.04^{b}	1.20 ± 0.04^{c}	1.24 ± 0.03^{bc}
Specific growth rate (SGR; %/fish/day)	0.83 ± 0.02^{c}	0.95 ± 0.02^{b}	1.04 ± 0.03^{a}	1.02 ± 0.02^{ab}
ADG	0.50 ± 0.01^{c}	0.61 ± 0.02^{b}	0.68 ± 0.03^{a}	0.66 ± 0.01^{ab}
initial number	20.00 ± 0.00	20.00 ± 0.00	20.00 ± 0.00	20.00 ± 0.00
Final number	18.33 ± 0.33^{b}	19.33 ± 0.33^{ab}	19.67 ± 0.33^{a}	19.33 ± 0.33^{ab}
Fish biomass per m3	1414.97 ± 35.67^{b}	1611.00 ± 13.26^{a}	1728.30 ± 47.78^{a}	1677.50 ± 33.83^{a}
Survival rate	91.67 ± 1.67^{b}	96.67 ± 1.67^{ab}	98.33 ± 1.67^{a}	96.67 ± 1.67^{ab}

Table 3
Effects of some probiotics on the body composition of Nile tilapia (O. niloticus).

Parameters	Control	T1	T2	T3
Dry matter	25.35 ± 0.05^{c}	27.04 ± 0.04^{b}	27.21 ± 0.04^{a}	27.33 ± 0.02^{a}
Crude protein	57.21 ± 0.51^{c}	59.60 ± 0.11^{b}	60.75 ± 0.04^{a}	60.99 ± 0.07^{a}
Ether Extract	25.34 ± 0.03^{a}	24.39 ± 0.02^{b}	24.27 ± 0.05^{c}	24.17 ± 0.03^{c}
Ash	14.45 ± 0.01^{d}	15.14 ± 0.02^{c}	15.42 ± 0.05^{b}	15.54 ± 0.02^{a}

Table 4
Effects of some probiotics on the biochemical markers of Nile tilapia (O. niloticus).

Parameters	Control	T1	T2	T3
AST (IU/L)	79.11 ± 0.04^{a}	78.22 ± 0.21^{a}	76.56 ± 0.11^{ab}	71.89 ± 0.18^{b}
ALT (IU/L)	8.34 ± 0.06^{a}	8.08 ± 0.02^{b}	7.92 ± 0.01^{c}	7.67 ± 0.05^{d}
ALP (IU/L)	31.45 ± 0.04^{a}	31.25 ± 0.06^{a}	30.73 ± 0.13^{b}	30.38 ± 0.05^{c}
Creatinine (mg/dL)	0.37 ± 0.01^{a}	0.36 ± 0.00^{a}	0.37 ± 0.00^{a}	0.37 ± 0.01^{a}
Urea (mg/dL)	4.55 ± 0.01^{a}	4.49 ± 0.02^{b}	4.45 ± 0.03^{bc}	4.41 ± 0.01^{c}
Albumin (g/dL)	1.44 ± 0.02^{d}	1.69 ± 0.02^{c}	1.90 ± 0.01^{b}	2.03 ± 0.02^{a}
Globulin (g/dL)	1.75 ± 0.01^{d}	2.02 ± 0.03^{c}	2.12 ± 0.01^{b}	2.20 ± 0.02^{a}
Total protein (g/dL)	3.20 ± 0.04^{b}	3.70 ± 0.05^{ab}	4.43 ± 0.43^{a}	4.23 ± 0.04^{a}

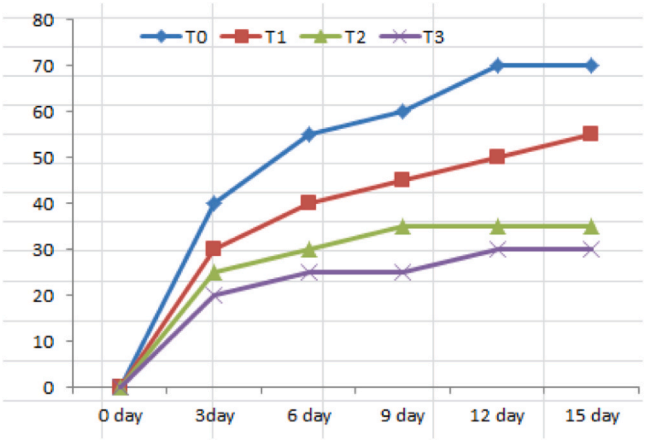

Fig. 1. Mortality of Nile tilapia (*Oreochromis niloticus*) fingerlings after infection *Aeromonas sobria* infection.

content seen in this study could be attributed to more proteins released by probiotics and efficient conversion of consumed food into structural protein, resulting in increased muscle development [50]. Fish administered with high dosages of probiotics had a lower ether extract composition. Yones, S Hussein, W Ali, M Abdel-Azem and Fisheries [51] and Eissa, Baghdady, Gaafar, El-Badawi, Bazina, Abd Al-Kareem and Abd El-Hamed [8] found similar results. In the current study, body composition analysis reveals that addition of probiotics to water of fish increased dry matter and ash contents compared to the control group. The present results are in parallel with [43] who found that inclusion of probiotics significantly increased dry matter and ash content of body composition.

In this study, probiotic could reduce AST, ALT and ALP enzymes, these results are in agreement with [41]. Meanwhile, Banerjee and Ray [52] found that probiotics had no effect on AST, ALT and ALP enzymes. In comparison to the control group, the usage of probiotics reduced urea levels. On the other hand, creatinine levels did not differ between groups, which is consistent with Moaheda, Alaryani, Elbahnaswy, Khattab, Elfeky, AbouelFadl, Eissa, Ahmed, Van Doan and El-Haroun [17]. Albumin is the most abundant protein in vertebrate blood plasma, acting as a carrier for a range of nutrients and metabolites [53]. Furthermore, larger amounts of serum protein, albumin, and globulins in fish were assumed to be associated with a strong innate immune response [54] [54].

When compared to the control group, probiotics added to water significantly reduced Nile tilapia mortality after A. sobria injection. According to these findings, probiotic improved the immune system of Nile tilapia, boosting the fish's resistance to A. sobria infection. This is consistent with Abou-El-Atta, Abdel-Tawwab, Abdel-Razek and Abdelhakim [55]. This is because probiotic bacteria can produce "bacteriocins" that are poisonous to other bacteria [46]. According to the findings of the above-mentioned study, probiotics significantly enhanced fish tolerance to many dangerous microbes [56].

5. Conclusion

This study exhibited the efficacy of water additive probiotic, as probiotic improved water quality, growth performance, feed utilization, biochemical analyses and disease resistance against *Aeromonas sobria* of

Nile tilapia (*Oreochromis niloticus*). As a result, water additive probiotics could be employed in sustainable aquaculture as a safe and novel alternative capable of improving growth, nutritional efficiency, and overall health in farmed fish.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] Bartley D. World Aquaculture 2020–A Brief Overview. FAO Fisheries and Aquaculture Circular, FAO; 2022. p. 1233.
[2] Xia Y, Wang M, Gao F, Lu M, Chen G. Effects of dietary probiotic supplementation on the growth, gut health and disease resistance of juvenile Nile tilapia (Oreochromis niloticus). Anim Nutr 2020;6(1):69–79.
[3] FAO, The state of world fisheries and aquaculture 2020. Sustainability in action. Rome., (2020).
[4] Behera BK, Pradhan PK, Swaminathan TR, Sood N, Paria P, Das A, Verma DK, Kumar R, Yadav MK, Dev AK, Parida PK, Das BK, Lal KK, Jena JK. Emergence of Tilapia Lake Virus associated with mortalities of farmed Nile Tilapia Oreochromis niloticus (Linnaeus 1758) in India. Aquaculture 2018;484:168–74.
[5] Eissa E-SH, El-Sayed A-FM, Ghanem SF, Dighiesh HS, Abd Elnabi HE, Hendam BM, Elleithy AA, Eissa MEH, Abd El-Aziz YM. Dietary mannan-oligosaccharides enhance hematological and biochemical parameters, reproductive physiology, and gene expression of hybrid red tilapia (Oreochromis niloticus x O. mossambicus). Aquaculture 2024;581:740453.
[6] Yasin R, Samiullah K, Fazal RM, Hussain S, Mahboob S, Al-Ghanim KA, Al-Misned FA, Ahmed Z. Combined effect of probiotics on prolonging the shelf life of GIFT tilapia fillets. Agricult Res 2020;51(12):5151–62.
[7] S.M. Limbu, L.-Q. Chen, M.-L. Zhang, Z.-Y. Du, A global analysis on the systemic effects of antibiotics in cultured fish and their potential human health risk: a review, 13(2) (2021) 1015–1059.
[8] Eissa E-SH, Baghdady ES, Gaafar AY, El-Badawi AA, Bazina WK, Abd Al-Kareem OM, Abd El-Hamed NNB. Assessing the influence of dietary pediococcus acidilactici probiotic supplementation in the feed of European sea bass (Dicentrarchus labrax L.) (Linnaeus, 1758) on farm water quality, growth, feed utilization, survival rate, body composition, blood biochemical parameters, and intestinal histology. Aquac Nutr 2022;2022:5841220.
[9] Eissa E-SH, Ahmed RA, Abd Elghany NA, Elfeky A, Saadony S, Ahmed NH, Sakr SE-S, Dayrit GB, Tolenada CPS, Atienza AAC, Mabrok M, Ayoub HF. Potential symbiotic effects of -1,3 glucan, and fructooligosaccharides on the growth performance, immune response, redox status, and resistance of pacific white shrimp, litopenaeus vannamei to fusarium solani. Infection 2023;8(2):105.
[10] Eissa E-SH, Elbahnaswy S, El-Baz AH, El-Haroun E, Ashour M, Mansour AT, Saadony S, El-Saeed AF, Kabary H, Jastaniah SD, Alaidaroos BA, Shafi ME, Abd El-Hack ME, Eissa MEH, Eldessouki EA. Effects of dietary commercial phytobiotic "Sanacore® GM" on Pacific white shrimp (Litopenaeus vannamei) growth, immune response, redux status, intestinal health, and disease resistance against Fusarium solani. Aquac Int 2023.
[11] Hendam BM, Munir MB, Eissa MEH, El-Haroun E, Doan Hv, Chung TH, Eissa E-SH. Effects of water additive probiotic, Pediococcus acidilactici on growth performance, feed utilization, hematology, gene expression and disease resistance against Aspergillus flavus of Nile tilapia (Oreochromis niloticus). Anim Feed Sci Technol 2023;303:115696.
[12] El-Saadony MT, Alagawany M, Patra AK, Kar I, Tiwari R, Dawood MAO, Dhama K, Abdel-Latif HMR. The functionality of probiotics in aquaculture: an overview. Fish Shellfish Immunol 2021;117:36–52.
[13] Monier MN, Kabary H, Elfeky A, Saadony S, El-Hamed NBA, Eissa MEH, Eissa E-SH. The effects of Bacillus species probiotics (Bacillus subtilis and B. licheniformis) on the water quality, immune responses, and resistance of whiteleg shrimp (Litopenaeus vannamei) against Fusarium solani infection. Aquac Int 2023;31(6):3437–55.
[14] Yilmaz S, Yilmaz E, Dawood MAO, Ringø E, Ahmadifar E, Abdel-Latif HMR. Probiotics, prebiotics, and synbiotics used to control vibriosis in fish: a review. Aquaculture 2022;547:737514.
[15] Jahangiri L, Esteban MÁJF. Administration of probiotics in the water in finfish aquaculture systems. a Rev 2018;3(3):33.
[16] Singh A, PavanKalyan M, Choudhury TG, Kamilya D, Khan MIR, Chouhan N. Supplementation of autochthonous potential probiotic Bacillus subtilis COFCAU_BSP3 to Labeo rohita feed: effect on immune-biochemical responses and resistance against Aeromonas hydrophila. Aquac Int 2023.
[17] Moaheda FSAlaryani, Elbahnaswy S, Khattab MS, Elfeky A, AbouelFadl KY, Eissa E-SH, Ahmed RA, Van Doan H, El-Haroun E. Dietary inclusion of Pediococcus acidilactici probiotic promoted the growth indices, hemato-biochemical indices, enzymatic profile, intestinal and liver histomorphology, and resistance of Nile Tilapia against Aspergillus flavus. Anim Feed Sci Technol 2023;306:115814.
[18] Cha J-H, Rahimnejad S, Yang S-Y, Kim K-W, Lee K-J. Evaluations of Bacillus spp. as dietary additives on growth performance, innate immunity and disease resistance of olive flounder (Paralichthys olivaceus) against Streptococcus iniae and as water additives. Aquaculture 2013;402-403:50–7.
[19] Hlordzi V, Kuebutornye FKA, Afriyie G, Abarike ED, Lu Y, Chi S, Anokyewaa MA. The use of Bacillus species in maintenance of water quality in aquaculture: a review. Aquac Rep 2020;18:100503.
[20] O. Khademzade, M. Zakeri, M. Haghi, S.M. Mousavi, The effects of water additive Bacillus cereus and Pediococcus acidilactici on water quality, growth performances, economic benefits, immunohematology and bacterial flora of whiteleg shrimp (Penaeus vannamei Boone, 1931) reared in earthen ponds, 51(5) (2020) 1759–1770.
[21] Eissa E-SH, Ahmed NH, El-Badawi AA, Munir MB, Abd Al-Kareem OM, Eissa MEH, Hussien EHM, Sakr SE-S. Assessing the influence of the inclusion of Bacillus subtilis AQUA-GROW® as feed additive on the growth performance, feed utilization, immunological responses and body composition of the Pacific white shrimp, Litopenaeus vannamei. Aquac Res 2022;53(18):6606–15.
[22] S. Mirzabekyan, N. Harutyunyan, A. Manvelyan, L. Malkhasyan, M. Balayan, S. Miralimova, M.L. Chikindas, V. Chistyakov, A. Pepoyan, Fish Probiotics: Cell Surface Properties of Fish Intestinal Lactobacilli and Escherichia coli, 11(3) (2023) 595.
[23] Ahmadifar E, Sadegh TH, Dawood MAO, Dadar M, Sheikhzadeh N. The effects of dietary Pediococcus pentosaceus on growth performance, hemato-immunological parameters and digestive enzyme activities of common carp (Cyprinus carpio). Aquaculture 2020;516:734656.
[24] Zibiene G, Zibas A. Impact of commercial probiotics on growth parameters of European catfish (Silurus glanis) and water quality in recirculating aquaculture systems. Aquac Int 2019;27(6):1751–66.
[25] Thurlow CM, Williams MA, Carrias A, Ran C, Newman M, Tweedie J, Allison E, Jescovitch LN, Wilson AE, Terhune JS, Liles MR. Bacillus velezensis AP193 exerts probiotic effects in channel catfish (Ictalurus punctatus) and reduces aquaculture pond eutrophication. Aquaculture 2019;503:347–56.
[26] Talpur AD, Munir MB, Mary A, Hashim R. Dietary probiotics and prebiotics improved food acceptability, growth performance, haematology and immunological parameters and disease resistance against Aeromonas hydrophila in snakehead (Channa striata) fingerlings. Aquaculture 2014;426-427:14–20.
[27] Li Y, Cai S-H. Identification and Pathogenicity of Aeromonas sobria on Tail-rot Disease in Juvenile Tilapia Oreochromis niloticus. Curr Microbiol 2011;62(2):623–7.
[28] J.-H. Yu, J.-J. Han, H.-J. Kim, S.-G. Kang, S.-W.J.Jofp Park, First report of Aeromonas veronii infection in farmed Israeli carp Cyprinus carpio in Korea, 23(2) (2010) 165–176.
[29] Noga EJ. Fish Disease: Diagnosis and Treatment. John Wiley & Sons; 2010.
[30] Moustafa EM, Dawood MAO, Assar DH, Omar AA, Elbialy ZI, Farrag FA, Shukry M, Zayed MM. Modulatory effects of fenugreek seeds powder on the histopathology, oxidative status, and immune related gene expression in Nile tilapia (Oreochromis niloticus) infected with Aeromonas hydrophila. Aquaculture 2020;515:734589.
[31] Ibrahim RE, Amer SA, Farroh KY, Al-Gabri NA, Ahmed AI, El-Araby DA, Ahmed SAA. The effects of chitosan-vitamin C nanocomposite supplementation on the growth performance, antioxidant status, immune response, and disease resistance of Nile tilapia (Oreochromis niloticus) fingerlings. Aquaculture 2021;534:736269.
[32] Cai W-q, Li S-f, Ma J-y. Diseases resistance of Nile tilapia (Oreochromis niloticus), blue tilapia (Oreochromis aureus) and their hybrid (female Nile tilapia × male blue tilapia) to Aeromonas sobria. Aquaculture 2004;229(1):79–87.
[33] Boyd CE. Water Quality: an Introduction. Springer Nature,; 2019.
[34] Chemists A.A.; Horwitz W., Official methods of analysis., AOAC, Arlington Vol. I. 15th ed. AOAC, Arlington, VA 489. (1990).
[35] Cunniff P. Official Methods of Analysis of the AOAC. sixteenth ed. Marylend: AOAC Internation; 1999.
[36] D. Heinegård, G.J.Cca Tiderström, Determination of serum creatinine by a direct colorimetric method, 43(3) (1973) 305–310.
[37] F.W. Sunderman Jr, M.W. Johnson, Studies of the serum proteins. 7. Sucrose gradient electrophoresis, 45 (1966) 381–397.
[38] Reitman S, Frankel SJA. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. 1957;28(1):56–63.
[39] W. Schäperclaus, H. Kulow, K.J. Schreckenbach, Fish diseases, (1992).
[40] Khalil SR, Reda RM, Awad A. Efficacy of Spirulina platensis diet supplements on disease resistance and immune-related gene expression in Cyprinus carpio L. exposed to herbicide atrazine. Fish Shellfish Immunol 2017;67:119–28.
[41] A. Kurdomanov, I. Sirakov, S. Stoyanova, K. Velichkova, I. Nedeva, Y.J.A. Staykov, Aquarium, Conservation, Legislation, The effect of diet supplemented with Proviotic® on growth, blood biochemical parameters and meat quality in rainbow trout (Oncorhynchus mykiss) cultivated in recirculation system, 12(2) (2019) 404–412.
[42] A.J.PotN.S. Fleck, Clinical and nutritional aspects of changes in acute-phase proteins during inflammation, 48(3) (1989) 347–354.
[43] Eissa E-SH, Ahmed NH, El-Badawi AA, Munir MB, Abd Al-Kareem OM, Eissa MEH, Hussien EHM, Sakr SE-S. Assessing the influence of the inclusion of Bacillus subtilis AQUA-GROW® as feed additive on the growth performance, feed utilization, immunological responses and body composition of the Pacific white shrimp. Litopenaeus Vannamei 2022;53(18):6606–15.
[44] Huerta-Rábago JA, Martínez-Porchas M, Miranda-Baeza A, Nieves-Soto M, Rivas-Vega ME, Martínez-Córdova LR. Addition of commercial probiotic in a biofloc shrimp farm of Litopenaeus vannamei during the nursery phase: Effect on bacterial diversity using massive sequencing 16S rRNA. Aquaculture 2019;502:391–9.
[45] Hossain M, Kamal M, Mannan M, Bhuyain MJR. Effects of probiotics on growth and survival of shrimp (Penaeus monodon) in coastal pond at Khulna, Bangladesh. 2013;5(2).
[46] Abuljadayel D. A BDA, Eissa MEH, Munir MB, Chowdhury AJ, Eissa EH. Utilization of probiotic bacteria Pediococcus acidilactici to enhance water quality, growth

performance, body composition, hematological indices, biochemical parameters, histopathology and resistance of red tilapia (Oreochromis sp.) against Aeromonas sobria. Desalin Water Treat 2023;1–8.
[47] Y. Deng, M.C. Verdegem, E. Eding, F.J.A. Kokou, Effect of rearing systems and dietary probiotic supplementation on the growth and gut microbiota of Nile tilapia (Oreochromis niloticus) larvae, 546 (2022) 737297.
[48] H. Van Doan, E. Wangkahart, W. Thaimuangphol, P. Panase, N.J.P. Sutthi, A. Proteins, Effects of Bacillus spp. mixture on growth, immune responses, expression of immune-related genes, and resistance of Nile Tilapia against Streptococcus agalactiae infection, (2021) 1–16.
[49] F.A. Salama, A. Abdel-Rahman, M.J.S. El Shehedy, Effect of adding Pediococcus acidilactici at low plant protein diets on growth performance of Nile tilapia (Oreochromis niloticus) fingerlings, 16(1) (2020) 93–105.
[50] M. Lara-Flores, M.A.R. Olvera-Novoa, The use of lactic acid bacteria isolated from intestinal tract of Nile tilapia (Oreochromis niloticus), as growth promoters in fish fed low protein diets, 41(3) (2013) 490–497.
[51] A.-M. Yones, M.S. Hussein, M.W. Ali, A.M. Abdel-Azem, Fisheries, Effect of dietary Lacto cel-con probiotic on growth performance and hematology indices of fingerlings mono-sex Nile tilapia (Oreochromis niloticus), 23(2) (2019) 227–239.
[52] Banerjee G, Ray AK. The advancement of probiotics research and its application in fish farming industries. Res Vet Sci 2017;115:66–77.
[53] E. Azzazy, R.H. Christenson, All About Albumin: Biochemistry, Genetics, and Medical Applications. Theodore Peters, Jr. San Diego, CA: Academic Press, 1996, 432 pp, $85.00. ISBN 0–12-552110–3, Oxford University Press, 1997.
[54] Wiegertjes GF. Immunogenetics of Disease Resistance in Fish. Wageningen University and Research; 1995.
[55] M.E. Abou-El-Atta, M. Abdel-Tawwab, N. Abdel-Razek, T.M.N. Abdelhakim, Effects of dietary probiotic Lactobacillus plantarum and whey protein concentrate on the productive parameters, immunity response and susceptibility of Nile tilapia, Oreochromis niloticus (L.), to Aeromonas sobria infection, 25(6) (2019) 1367–1377.
[56] S.H. Hoseinifar, Y.-Z. Sun, A. Wang, Z.J.Fim Zhou, Probiotics as means of diseases control in aquaculture, a review of current knowledge and future perspectives, 9 (2018) 2429.