Desalination and Water Treatment
www.deswater.com

doi: 10.5004/dwt.2023.30013

315 (2023) 469–478
December

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*

Dalia A. Abuljadayel[a], Duaa Abdulrashid Abdulghani Bukhari[b], Moaheda E.H. Eissa[c], Mohammad Bodrul Munir[d,*], Ahmed Jalal Khan Chowdhury[d,*], El-Sayed Hemdan Eissa[e,*]

[a]*Department of Biological Science, College of Science, King Abdulaziz University, Jeddeh, Saudi Arabia*
[b]*Applied College, Taibah University, Medinah, Saudi Arabia*
[c]*Biotechnology Department, Fish Farming and Technology Institute, Suez Canal University*
[d]*Faculty of Agriculture, Sinaut Campus, Universiti Islam Sultan Sharif Ali, Tutong TB1741, Brunei Darussalam, email: hsjewel730@yahoo.com (M.B. Munir), dhaka89@hotmail.com (A.J.K. Chowdhury)*
[e]*Fish Resources and Aquaculture Department, Faculty of Agricultural Environmental Sciences, Arish University, Egypt, email: Sayed.Hemdan@env.aru.edu.eg*

ABSTRACT

Aquaculture uses probiotic bacteria as a microbiological supplement in place of antibiotics. The current study's objective was to determine how well red tilapia (*Oreochromis* sp.) raising water responded to *Pediococcus acidilactici* as a probiotic bacterium. For the four groups (one control group, three test groups), a total of 120 fingerlings (13.00 ± 0.10 g) were used. *P. acidilactici* was treated in growth water in three test groups at various concentrations. In terms of ultimate body weight, weight increase, and specific growth rate, fish raised in water supplemented at a high level with *P. acidilactici* performed noticeably better during growth compared to the control. When fish rearing water included lower levels of *P. acidilactici* than the control group, feed conversion ratios were considerably improved. In comparison to the untreated group, the range and median levels of unionized ammonia in water were likewise significantly reduced by probiotic water additives. When compared to the control group, the treated tilapia water with a high concentration of *P. acidilactici* had higher amounts of crude protein and ash as well as lower levels of fat. The heights of the intestinal villi increased as the water's probiotic content rose. Finally, red tilapia fish were experimentally challenged with pathogenic *Aeromonas sobria*, and mortalities were daily recorded for 15 d following infection, indicating the effect of probiotics feeding to enhance resistance to *A. sobria*.

Keywords: *Pediococcus acidilactici*; *Oreochromis* sp.; Water quality; Growth performance; body composition; biochemical parameters, Histology; *Aeromonas sobria*

1. Introduction

Aquaculture is still a major source of food and income for millions of people around the world. Ailments and unfavorable environmental factors continually reduce production, resulting in significant economic losses for farming [1,2]. Poor management practises in the aquaculture industry, such as overfeeding, high stock densities, destructive fishing techniques, and polluted water, increase the chance of illness signs [3,4]. Since there are concerns about antibiotic overuse and antibiotic residues in aquatic animals, fish producers only resort to antibiotics as a last resort to address a variety of illnesses [5,6]. Antibiotics are often not assured to be successful since bacteria may develop resistance to them after use, which is detrimental for the environment

* Corresponding authors.

[7–10]. Nonetheless, the use of prebiotics and probiotics in aquaculture has lately increased [11–14].

The lactic acid bacteria, the yeast *Saccharomyces boulardii*, and the enterococci are the probiotics that are most frequently employed [15,16]. Due to their distinct physiological, morphological, and metabolic characteristics, as well as their capacity to secrete a variety of enzymes and a health-promoting organic acid and aromatic compounds. They secrete peptides that are harmful to pathogens but not to the host, known as antimicrobial peptides [17]. A gram-positive bacteria called *Pediococcus acidilactici* selectively ferments lactic acid (LAB) and can endure a variety of acidities [18–20]. These helpful bacteria boost feed consumption and gut microbiota diversification, two processes that are frequently exploited as growth promoters in a range of aquatic animals [21–23]. In addition, several descriptions of *P. acidilactici* probiotics concentrate on boosting immune function and enhancing fish species' tolerance to infectious diseases [24–26].

The common freshwater cultivated fish known as tilapia (Nile, Red and other) can be raised in a variety of habitats and will consume a variety of feed ingredients. Tilapia cultivation produces 5% of all finfish aquaculture and 75% of all aquaculture worldwide [27]. There is a connection between the growth of tilapia fish aquaculture and serious infections [28]. Fish are continuously under stress due to overstocking and poor water quality, which increases their susceptibility to disease [29,30]. Many wild and farmed fish species, including tilapia (*Oreochromis* sp.), and perch (*Perca fluviatilis*), have been found to be infected by *Aeromonas sobria* [7,31]. Septicemia, fin and tail rot, and ulcerative dermatitis are among the clinical indications displayed by fish infected with *A. sobria* [32]. This pathogen can infect both humans and other vertebrates, and in those who have poor immune systems, this can lead to serious local and systemic infections [33]. Hence, the goal of this study was to see if using the probiotic bacteria *P. acidilactici* may boost red tilapia fish (*Oreochromis* sp.) resistance to *A. sobria* by improving water quality, growth performance, body composition, haematological indices, biochemical parameters, and gut histology.

2. Materials and methods

2.1. Experimental approach

The fish was divided into four experimental groups which are: T0: with the addition of 0.0 g *P. acidilactici* per m^3 water; T1: with the addition of 0.1 g *P. acidilactici* per m^3 water; T2: with the addition of 0.2 g *P. acidilactici* per m^3 water; T3: with the addition of 0.3 g *P. acidilactici* per m^3 water. Commercial probiotics (Bactocell PA10) were applied which contained encapsulated lactic acid bacteria (*P. acidilactici*). Fish were given commercial diets three times per day at 07:00, 11:00, and 15:00 at a rate determined by fish biomass (5% in the first 28 d, 4% in the second 28 d, and 3% in the last 14 d in all groups). Fingerlings were fed on the extruded diets of Aller Aqua Company (Cairo, Egypt) (https://www.aller-aqua.com/). The proximate chemical composition of the used commercial diet is 30% protein, crude lipid (5.2%), fiber (4.8%), NFE (47.2%), ash (5.8%). Each experimental group's fish were live-weighed every two weeks in order to estimate the quantity of feed ingested throughout the trial period. There is simply natural sunlight.

In the three experimental probiotic treatments, 10% of the water was changed every day to account for the probiotics that would be lost during water changes and to provide 10% extra probiotics each day. For the probiotic-free control treatments, the water was switched by 25% each day to investigate how *P. acidilactici* affected the performance of red tilapia fish and to improve the test aquariums' water quality characteristics by reducing the rate of water changes. Probiotics from Bactocell PA10 were used in three different dosages.

2.1.1. Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of fish.

2.2. Testing water quality

Every day at 3 pm, water quality parameters were measured. An oxygen meter with temperature and oxygen probes was used to measure temperature and dissolved oxygen (DO) levels. The pH readings were taken using a pH meter. A refractometer (Erma, Japan) was used to assess the water's salinity [13]. The HANNA HI-96715-11 ammonia medium range photometer was used to measure the total ammonia nitrogen (TAN) (HANNA, Nusfalau, Romania).

2.3. Evaluation for fish growth

Fish specimens were taken and weighed separately to determine the growth and feed utilization variables. The criteria investigated included body weight gain (WG), weight gain rate (WG%), specific growth rate (SGR), survival rate (SR), feed intake (FI), and feed conversion ratio (FCR) according to the study of Sharifah et al. [34].

2.4. Body's overall composition

Analyses of diet and fish body composition were carried out using conventional methods [35]. The fish was autoclaved, then homogeneously mashed into a liquid, dried in an oven, and then reground before analysis. A Kjeltec auto analyzer (Model 1030, Tecator, Hgans, Sweden) was used to calculate the amounts of crude protein. Crude lipid concentrations were estimated according to the Bligh and Dyer [36]. Standard procedures were used to analyze the contents of moisture, dry matter, and ash [37].

2.5. Collection of blood

Nine fish from each treated group-three fish in each aquarium—were randomly sampled after a 24-h fast. According to Adeshina et al. [38] the fish was put to sleep for 3 min with 95 mg/L clove oil before blood samples were taken from the caudal vein with a 1-mL sterile syringe. In order to identify antioxidant and hematological markers, the pooled blood specimen was combined with dipotassium salt of ethylenediaminetetraacetic acid as an anticoagulant (0.5 mg/mL blood), transferred to Eppendorf tubes, and centrifuged (3,000 g for 15 min). Until it was required, the serum was maintained at 20°C.

2.6. Blood biochemical analysis

According to Stoskopf [39], red blood cells (RBC) were counted using a hemocytometer and Natt & Herrick's solution. After adding Drabkin's solution, the haemoglobin was measured using the study of Blaxhall and Daisley [40]. Several researchers assessed the total protein, albumin, and globulin contents of the collected serum [41–43]. Reitman and Frankel [44] tested the enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP). The formula developed by Heinegard and Tiderstrom was used to determine creatinine [45]. According to Trinder [46], serum glucose was measured using a "Boehringer" blood sugar testing kit.

2.7. Microscopic examination of intestinal sections

Three fish from each group were anaesthetized at random with 40% ethyl alcohol after 70 d of feeding [19]. Fish intestines were removed and divided into proximal, middle, and distal sections [47]. After the esophageal aperture, the proximal section continues till the start of the hepatic loop. The proximal major coil, the stomach loop, and the distal major coil make up the central portion of the intestine that is coiled. After the coiled segment, the distal or terminal portion of the intestine starts and reaches up to 2 cm before the anus. About 1 cm long segments of the intestine were fixed in Bouin's solution for the night before being moved to 70% ethanol. Before being embedded in paraffin, the samples underwent an increasing alcohol series (70%–100%) of dehydration. After being transversely divided into 5 mm-thick sections, the intestinal portions were stained with hematoxylin and eosin (H&E). With a light microscope and a camera, the sections were analyzed. The length, and width of the intestinal villi as well as muscular thickness in each region were measured using Image J version 1.36.

2.8. A. sobria challenge test

A pathogenic *A. sobria* strain was applied. After a 70-d of experiment, the resistance of red tilapia to pathogenic *A. sobria* was investigated, which was grown in an incubator on nutrient broth for 24 h at 30°C. Bacterial pellets were obtained through centrifugation at 3,000 g for 30 min. The pellets were suspended in 1.0 mL of 0.1% peptone water and inoculated with a 0.1 mL dose of 24 h broth from *A. sobria* (5×10^5 CFU/mL) as described by Schäperclaus [48]. Intraperitoneal, 1.5×10^7 cells/mL cell suspension was injected into the fish [49]. For 15 d the challenged fish were observed daily to record mortalities and/or clinical signs.

2.9. Statistical analysis

The results were statistically tested in SPSS version by the one-way analysis of variance (ANOVA) test. Differences were considered significant at ($P < 0.05$). Polynomial regression analysis was used on growth performance parameters and feed conversion ratio to determine the linear effects of probiotic supplementation on the tested variables [50].

3. Results

3.1. Water quality

In the current experiment, which used a regulated photoperiod (12 h of light followed by 12 h of darkness), all water quality values fell within the appropriate limits for red tilapia rearing: Temperature, dissolved oxygen, TAN, salinity, and pH were all measured. When contrasted with the control group, probiotic water additives significantly reduced the TAN values ($P \leq 0.05$) in water and enhanced the aquatic environment as depicted in Table 1.

3.2. Growth results

Table 2 displays how *P. acidilactici* additive impacted the growth performance of red tilapia fingerlings. When compared to the control group, the growth performance of *P. acidilactici* treatments was higher value as final weight (g/fish). FCR was higher with T3 than with other groups. The fish group fed T3, followed by T2 and T1, had the best weight gain, SGR, biomass, feed intake, and survival rate ($P > 0.05$), while the fish control group's rates considerably reduced.

3.3. Body composition

Table 3 shows the fish's total body composition after being raised in probiotic-infused water. In comparison to the control group, crude protein and ash concentrations increased in all treatment groups. The T3 group had the highest values, followed by the T2 and T1 groups, respectively. In contrast, compared to the control group, all probiotic supplemented groups showed lower lipid concentrations.

Table 1
Effect of *Pediococcus acidilactici* probiotic on water quality parameters during rearing of red tilapia (*Oreochromis* sp.) in a controlled photoperiod (12-h light:12-h darkness)

Parameters	Control	T1	T2	T3
Temperature (°C	27.365 ± 0.024	27.583 ± 0.082	27.173 ± 0.094	27.317 ± 0.148
Salinity (ppt)	215.667 ± 1.856	217.00 ± 0.577^b	216.667 ± 0.882^b	234.667 ± 0.882
DO2 (ppm)	8.013 ± 0.063	8.200 ± 0.076	7.683 ± 0.103^b	7.310 ± 0.126
pH	8.180 ± 0.009	8.173 ± 0.003	8.123 ± 0.015	8.123 ± 0.012
TAN (ppm)	0.943 ± 0.044^d	0.689 ± 0.003^c	0.532 ± 0.004^b	0.432 ± 0.002^a

Superscripts in each column represent significant ($P < 0.05$) differences between the biological replicates.

Table 2
Effect of *Pediococcus acidilactici* on the growth performance and feed utilization of red tilapia (*Oreochromis* sp.)

	Contol	T1	T2	T3
Initial fish weight (g)	13.00 ± 0.10[a]	13.07 ± 0.09[a]	13.00 ± 0.12[a]	13.10 ± 0.10[a]
Final fish weight (g)	39.80 ± 0.72[d]	44.43 ± 0.41[c]	46.63 ± 0.48[b]	48.33 ± 0.20[a]
Weight gain	26.80 ± 0.64[d]	31.37 ± 0.33[c]	33.63 ± 0.37[b]	35.23 ± 0.13[a]
Weight gain (%)	206.12 ± 3.85[d]	240.04 ± 1.25[c]	258.71 ± 1.10[b]	268.98 ± 1.76[a]
SGR	1.60 ± 0.02[d]	1.75 ± 0.01[c]	1.82 ± 0.00[b]	1.86 ± 0.01[a]
Feed intake	46.58 ± 0.72[c]	49.60 ± 0.34[b]	50.77 ± 0.36[ab]	51.51 ± 0.46[a]
FCR	1.74 ± 0.02[a]	1.58 ± 0.01[b]	1.51 ± 0.01[c]	1.46 ± 0.01[d]
ADG	0.39 ± 0.01[d]	0.45 ± 0.01[c]	0.48 ± 0.01[b]	0.50 ± 0.00[a]
Initial number	10.00 ± 0.00	10.00 ± 0.00	10.00 ± 0.00	10.00 ± 0.00
Final number	8.67 ± 0.33[b]	9.00 ± 0.58[ab]	10.00 ± 0.00[a]	9.67 ± 0.33[ab]
Survival rate (%)	86.67 ± 3.33[b]	90.00 ± 5.77[ab]	100.00 ± 0.00[a]	96.67 ± 3.33[ab]
Biomass per m^3	345.37 ± 18.90[b]	399.43 ± 22.06[b]	466.33 ± 4.81[a]	467.23 ± 16.39[a]

Superscripts in each column represent significant ($P < 0.05$) differences between the biological replicates.

Table 3
Impacts of *Pediococcus acidilactici* probiotic on the body composition of red tilapia (*Oreochromis* sp.)

Parameters	Control	T1	T2	T3
DM (%)	25.82 ± 0.13[c]	27.02 ± 0.27[b]	27.27 ± 0.15[b]	28.19 ± 0.15[a]
Protein (%)	57.64 ± 0.10[c]	59.54 ± 0.15[b]	60.81 ± 0.23[a]	60.92 ± 0.12[a]
Ether Extract (%)	25.39 ± 0.20[a]	24.78 ± 0.13[b]	23.28 ± 0.06[c]	22.99 ± 0.07[c]
Ash (%)	14.54 ± 0.05[d]	15.47 ± 0.08[c]	15.74 ± 0.05[b]	16.00 ± 0.06[a]

Superscripts in each column represent significant ($P < 0.05$) differences between the biological replicates.

Table 4
Effects of *Pediococcus acidilactici* on hematological and serum biochemical parameters of red tilapia (*Oreochromis* sp.)

Parameters	T0	T1	T2	T3
Albumin	1.37 ± 0.03[a]	1.59 ± 0.01[b]	1.61 ± 0.02[b]	1.81 ± 0.03[c]
Globulin	1.81 ± 0.02[a]	1.87 ± 0.01[a]	2.14 ± 0.08[b]	2.13 ± 0.05[b]
Total protein (mg/mL)	3.18 ± 0.04[a]	3.46 ± 0.01[b]	3.75 ± 0.08[c]	3.94 ± 0.05[d]
ALT (U/L)	47.76 ± 0.83[b]	46.45 ± 0.09[ab]	45.99 ± 0.19[a]	45.10 ± 0.04[a]
AST (U/L)	126.10 ± 2.06[c]	121.13 ± 0.30[b]	118.28 ± 0.29[ab]	117.18 ± 0.63[a]
ALP (U/L)	26.91 ± 0.21[d]	23.33 ± 0.18[c]	21.68 ± 0.04[b]	21.09 ± 0.03[a]
RBC (10^6 mm^{-3})	1.20 ± 0.01[a]	1.35 ± 0.01[b]	1.44 ± 0.01[c]	1.44 ± 0.01[c]
Hb (g/dl)	6.15 ± 0.02[a]	7.34 ± 0.06[b]	7.90 ± 0.01[c]	7.92 ± 0.01[c]
Hct (%((pcv)	27.33 ± 0.10[a]	28.30 ± 0.07[b]	28.78 ± 0.05[c]	28.87 ± 0.01[c]
MCV (fl)	230.86 ± 2.60[c]	209.66 ± 1.99[b]	197.34 ± 4.27[a]	200.47 ± 0.84[a]
MCH (pg)	51.26 ± 0.64[a]	54.35 ± 0.05[b]	54.97 ± 0.28[b]	55.02 ± 0.18[b]
MCHC (%)	23.09 ± 0.46[a]	25.92 ± 0.25[b]	27.44 ± 0.08[c]	27.45 ± 0.03[c]
Creatinine	61.76 ± 0.65[d]	56.42 ± 0.60[c]	51.79 ± 0.62[b]	49.38 ± 0.19[a]
glucose (mg/dl)	129.40 ± 0.73[c]	125.99 ± 0.13[b]	126.34 ± 0.25[b]	123.72 ± 0.35[a]

Superscripts in each column represent significant ($P < 0.05$) differences between the biological replicates.

3.4. Biochemical parameters

Results in Table 4 show that the RBCs and haemoglobin showed slight elevation in values in fish reared on probiotic. The addition of *P. acidilactici* decreased in AST, ALT, ALP and creatinine values compared with the control. Glucose activities slightly decreased in T1, T2 and T3 fish groups compared to the control group. The supplementation of probiotic showed a minor elevation in the serum total protein, albumin and globulin levels than in the control group after 70 d.

3.5. Examination of histological sections

The intestinal villi of the T2 and T3 groups were found to be branching and noticeably longer, with the T3 group exhibiting a minor degree of apical villous sloughing. The apical portion of the intestinal villi in the control group clearly sloughed off. The villus length, width and muscular thickness the red tilapia intestine slightly increased upon using *P. acidilactici* in larger doses as depicted in (Figs. 1 and 2).

3.6. A. sobria challenge test

Adding probiotic to water, dramatically increased ($P > 0.05$) red tilapia resistance to *A. sobria* infection compared to the control group as shown in (Fig. 3). Exophthalmia, ascites, tail and fin rots, scalelessness with external haemorrhage, and septicaemic lesions of the internal organs were found after the fish had died. Clinical indicators of death included exophthalmia as well as internal organ septicaemia.

4. Discussion

During routine inspection, it was found that the determined water quality parameters were within the range that was suitable for tilapia growth [51,52]. All living things, including cichlid fish species, depend on DO, a crucial aspect of water quality [53]. Moreover, fish and other aquatic species are stressed, prone to infectious illnesses, and grow slowly at low dissolved-oxygen levels (4 mg/L) [54]. According to the results, adding probiotics to fish water may have an impact on DO levels. Understanding the dynamics of TAN and other nitrogenous waste is essential for the sustainability of intensive aquaculture systems and preventing unexpected fish deaths [8,55]. The results show that tilapia water treatment can lower ammonia range levels and enhance the aquatic environment. These results were in line with those of Khademzade et al. [56], who found that adding two bacterial strains— *P. acidilactici* and *Bacillus cereus*- to a shrimp pond considerably lowered the nitrogenous concentration. Also, according to John et al. [57], adding probiotic strains to *Oreochromis mossambicus*' rearing water dramatically decreased the levels of ammonia. Several forms of nitrogen can be removed from fish wastewater by *Bacillus* species [58] and are crucial for controlling the nitrogen cycle through nitrogen fixation, nitrification, and denitrification as well as ammonification [59,60]. This research supports a report by Naiel et al. [55] indicating administration of various probiotic strains in rearing water improves the water quality, performance, body chemical analysis, antioxidant response, and immune responses of tilapia.

Addition of *P. acidilactici* to tilapia water improved weight gain, SGR, feed intake, FCR, biomass, and survival rate in comparison to water without *P. acidilactici*. It has

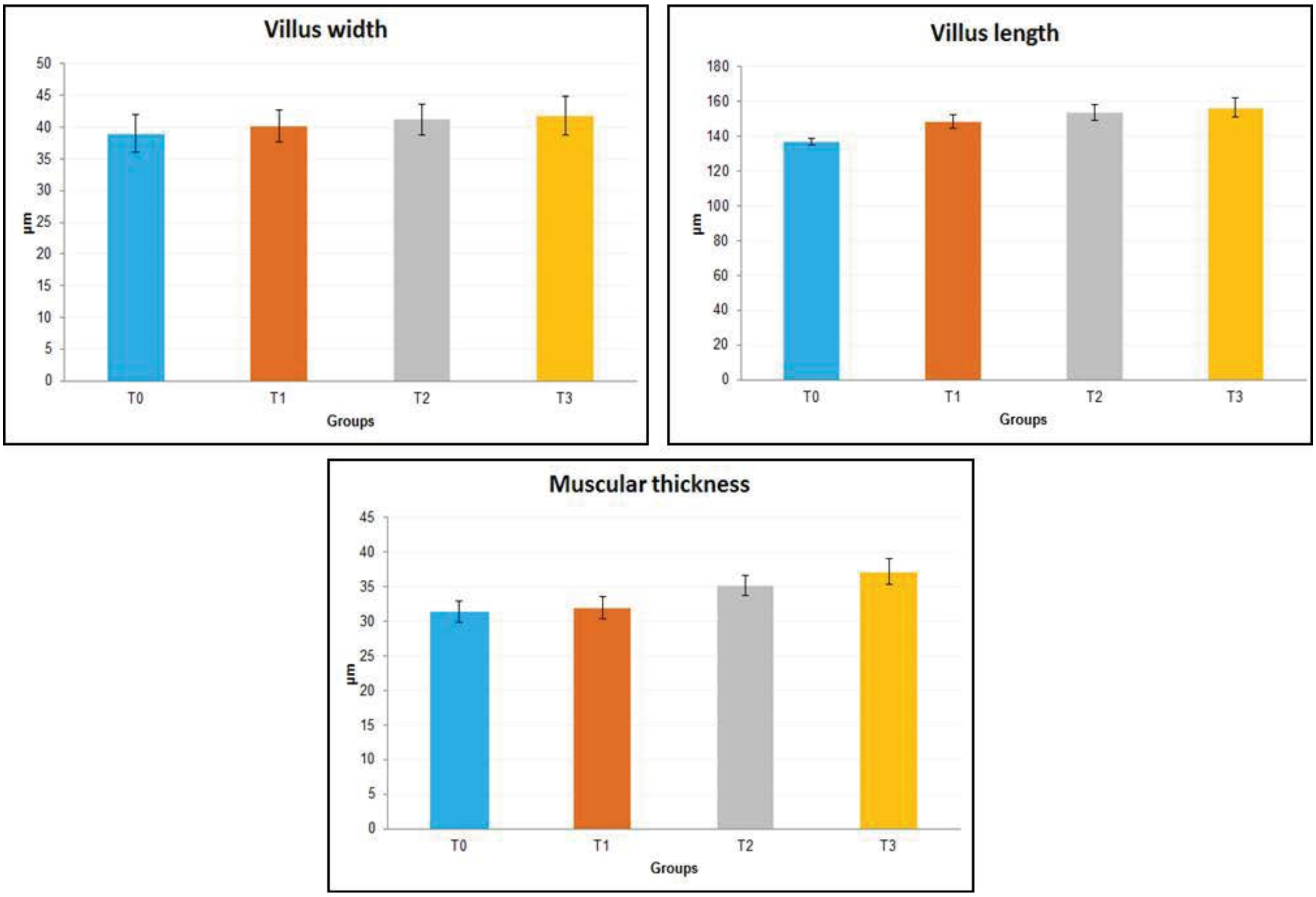

Fig. 1. Impact of probiotic diets on villus width, length and muscular thickness of red tilapia (Data are represented as means ± standard errors).

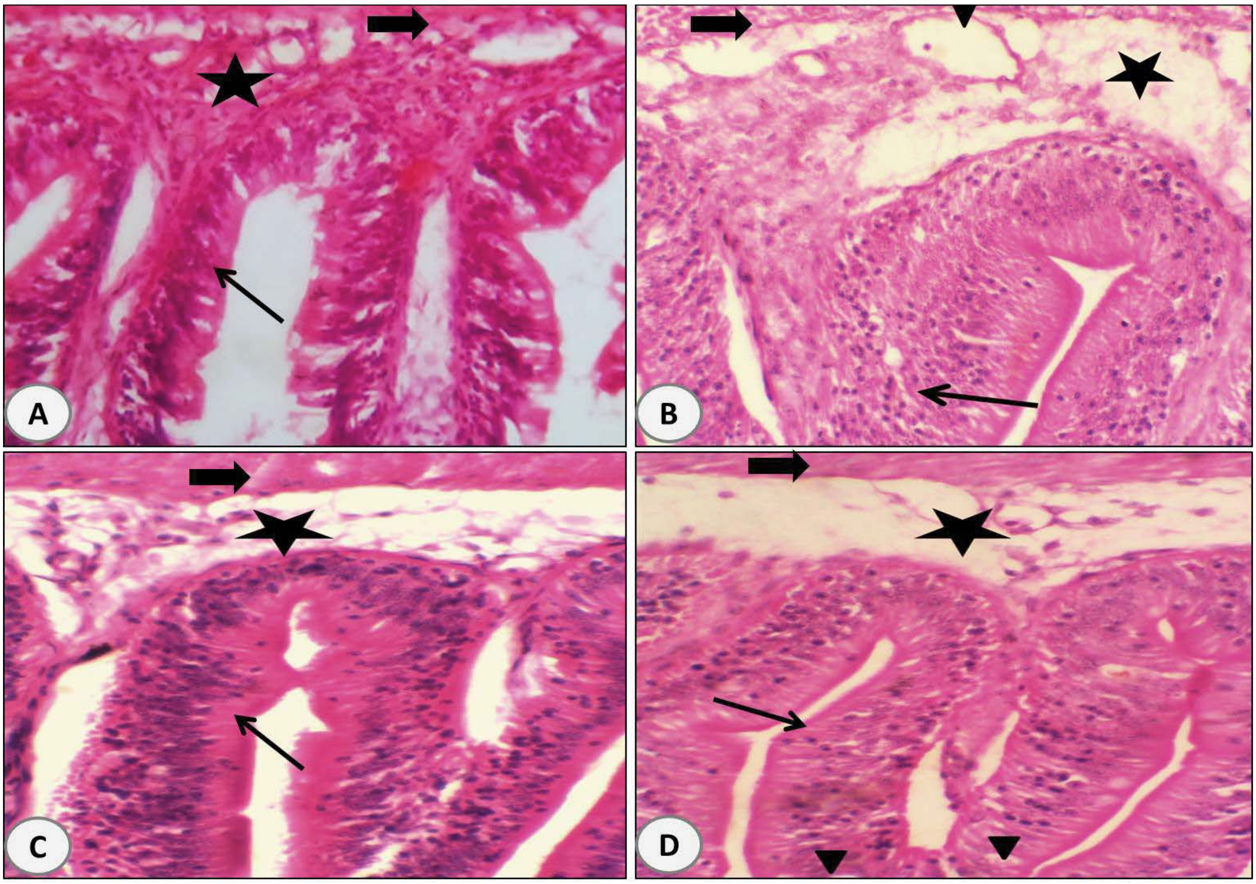

Fig. 2. Histological sections of red tilapia intestine fed with the experimental diets. (A) Control (T0), (B) T1, (C) T2, and (D) T3 (scale bar = 50 µm). Muscular layer (thick arrows) and villi (thin arrows), loose connective tissues (stars), and some vacuoles (arrowheads).

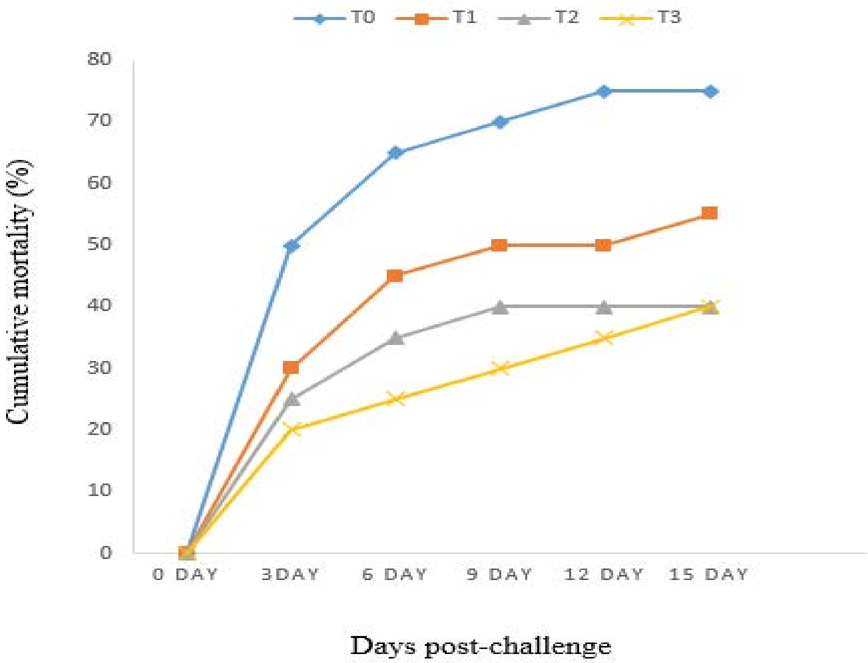

Fig. 3. Variations in the cumulative mortality (%) of tilapia fish (*Oreochromis* sp.) for 70 d and challenged with *Aeromonas sobria* for 15 d.

been reported that using probiotics in fish culture water had a positive effect on the growth and efficiency of consumed feed in tilapia. Early reports have also illustrated that probiotic-enriched tilapia rearing water dramatically enhanced growth and productivity [8,61–63]. *P. acidilactici* supplementation elevated the crude protein of *Oreochromis* sp. According to the findings, using *Streptococcus faecium* or *Lactobacillus acidophilus* improved the protein content of fishes [8,11]. This could be due to improvement of feed intake, improved digestibility and usage of nutrients, as well as variations in muscular deposition rate [64]. In contrast, probiotic-supplemented Senegalese sole and rabbit fish had no effect on body composition [65].

Biochemical parameters are beneficial in predicting fish health [66]. Probiotic-supplemented water used in this study increased RBC count and haemoglobin content. These findings suggest that using *P. acidilactici* in *Oreochromis* sp. was safe and capable of improving body status. Several investigators reported no significant differences in RBC counts after feeding *Bacillus* to tilapia and rainbow trout compared to controls [67,68].

Blood protein fraction such as total proteins (TP), which is composed of albumin and globulin, is frequently dignified as a pointer of aquatic animal immune and health profiles [69], and its elevation indicates a more robust innate immune eminence [70] and this is in agreement with our study.

Furthermore, the data revealed that T1, T2, and T3 had lower creatinine, ALT, and AST levels than the control. Furthermore, the inclusion rate of probiotics in their diets had an effect on their activity. The liver enzymes ALT and AST are essential indicators of liver health and function and this is in agreement with Eissa et al. [11].

The histology of the probiotic-treated tilapia in the current investigation showed noticeably more positive intestinal changes than those in the control group. The results of this study are in agreement with earlier studies on the advantages of probiotics [71]. Fish intestinal goblet cells have both secretory and absorptive activities [11]. In all areas of the gut, goblet cells dramatically expanded after *P. acidilactici* treatment. The gut epithelium is inhibited by gastrointestinal microbiota, which also reduces brush border activity, causes mucosal damage, and decreases absorption area. The gut epithelium is also inhibited by the absence of gastrointestinal microbiota, which also reduces brush border activity, causes mucosal damage, and decreases absorption area [72,73].

Aquaculture will only benefit from immunostimulants if they considerably increase fish protection against pathogenic bacterial infection. Disease resistance is frequently evaluated using fish survival after pathogen challenge [74]. Probiotics added to water considerably decreased red tilapia mortality after injection with *A. sobria* when contrasted with the control group. According to these results, *P. acidilactici* considerably enhanced the Nile tilapia's non-specific immune system, increasing the fish's resistance to *A. sobria* infection, This is in agreement with report by Abou-El-Atta et al. [75]. This is as a result of the probiotic bacteria's capacity to generate "bacteriocins" that are toxic to other bacteria, like *P. acidilactici* [76]. The results of the research indicated above showed that *P. acidilactici* considerably increased fish resistance to different harmful microorganisms [77]. According to Lee et al. [78], fish raised on probiotic *Bacillus subtilis* were considerably more resistant to bacterial invasion with *Vibrio anguillarum* than fish raised on the control group.

5. Conclusion

The use of *P. acidilactici* can enhance water quality, red tilapia fish growth performance, body composition, and resistance to *A. sobria*. A high concentration of *P. acidilactici* (0.3 g/m^3) in fish water produced the best results in improving *Oreochromis* sp. response's to *A. sobria* infection, and this can be considered as a potential candidate to replace the use of antibiotics in water.

Funding

No funding.

Data availability statement

All created data during this study were inserted in the manuscript.

Conflicts of interest

The authors declare no conflict of interest.

References

[1] E.-S.H. Eissa, R.A. Ahmed, N.A. Abd Elghany, A. Elfeky, S. Saadony, N.H. Ahmed, S. El-Sayed Sakr, G.B. Dayrit, C.P.S. Tolenada, A.A.C. Atienza, M. Mabrok, H.F. Ayoub, Potential symbiotic effects of β-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, Fishes, 8 (2023) 105, doi: 10.3390/fishes8020105.

[2] E.M. Okon, H.N. Birikorang, M.B. Munir, Z.A. Kari, G. Téllez-Isaías, N.E. Khalifa, S.A. Abdelnour, M.E.H. Eissa, A. Al-Farga, H.S. Dighiesh, E.-S. Hemdan Eissa, A global analysis of climate change and the impacts on oyster diseases, Sustainability, 15 (2023) 12775, doi: 10.3390/su151712775.

[3] E.-S.H. Eissa, W.K. Bazina, Y.M. Abd El-Aziz, N.A. Abd Elghany, W.A. Tawfik, M.I. Mossa, O.H. Abd El Megeed, N.N.B. Abd El-Hamed, A.F. El-Saeed, E. El-Haroun, S.J. Davies, O.J. Hasimuna, M.E.H. Eissa, H.S. Khalil, Nano-selenium impacts on growth performance, digestive enzymes, antioxidant, immune resistance and histopathological scores of Nile tilapia, *Oreochromis niloticus* against *Aspergillus flavus* infection, Aquacult. Int., (2023), doi: 10.1007/s10499-023-01230-4.

[4] E.-S.H. Eissa, O.H. Ezzo, H.S. Khalil, W.A. Tawfik, A.A. El-Badawi, N.A. Abd Elghany, M.I. Mossa, M.M. Hassan, M.M. Hassan, M.E.H. Eissa, M.E. Shafi, A.H. Hamouda, The effect of dietary nanocurcumin on the growth performance, body composition, haemato-biochemical parameters and histopathological scores of the Nile tilapia (*Oreochromis niloticus*) challenged with *Aspergillus flavus*, Aquacul. Res., 53 (2022) 6098–6111.

[5] E.-S.H. Eissa, B.A. Alaidaroos, S.D. Jastaniah, M.B. Munir, M.E. Shafi, Y.M. Abd El-Aziz, W.K. Bazina, S.b. Ibrahim, M.E.H. Eissa, M. Paolucci, F.S. Alaryani, N.N.B. Abd El-Hamed, M.E. Abd El-Hack, S. Saadony, Dietary effects of nano curcumin on growth performances, body composition, blood parameters and histopathological alternation in red tilapia (*Oreochromis* sp.) challenged with *Aspergillus flavus*, Fishes, 8 (2023) 208, doi: 10.3390/fishes8040208.

[6] K. Thornber, D. Huso, M.M. Rahman, H. Biswas, M.H. Rahman, E. Brum, C.R. Tyler, Raising awareness of antimicrobial resistance in rural aquaculture practice in Bangladesh through digital communications: a pilot study, Global Health Action, 12 (2019) 1734735, doi: 10.1080/16549716.2020.1734735.
[7] G.A. Coscelli, C. Casabonne, E. Morón-Alcain, N. Arancegui, F.A. Vigliano, *Aeromonas sobria*, an outbreak of natural infection in cultured silver catfish *Rhamdia quelen* (Quoy & Gaimard, 1824) in Argentina, J. Fish Dis., 40 (2017) 1929–1933.
[8] B.M. Hendam, M.B. Munir, M.E.H. Eissa, E. El-Haroun, H. van Doan, T.H. Chung, E.-S.H. Eissa, 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., 303 (2023) 115696, doi: 10.1016/j.anifeedsci.2023.115696.
[9] J.E.M. Watts, H.J. Schreier, L. Lanska, M.S. Hale, The rising tide of antimicrobial resistance in aquaculture: sources, sinks and solutions, Mar. Drugs, 15 (2017) 158, doi: 10.3390/md15060158.
[10] S. Won, A. Hamidoghli, W. Choi, J. Bae, W.J. Jang, S. Lee, S.C. Bai, Evaluation of potential probiotics *Bacillus subtilis* WB60, *Pediococcus pentosaceus*, and *Lactococcus lactis* on growth performance, immune response, gut histology and immune-related genes in Whiteleg Shrimp, *Litopenaeus vannamei*, Microorganisms, 8 (2020) 281, doi: 10.3390/microorganisms8020281.
[11] E.-S.H. Eissa, E.S. Baghdady, A.Y. Gaafar, A.A. El-Badawi, W.K. Bazina, O.M. Abd Al-Kareem, N.N.B. Abd El-Hamed, 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, Aquacult. Nutr., 2022 (2022) 5841220 (11 Pages), doi: 10.1155/2022/5841220.
[12] E.-S.H. Eissa, C.I. Che-Zulkifli, A.A. El-Badawi, MA.M. Ali, E.S. Baghdady, O.M. Abd Al-Kareem, R.A. Ahmed, Growth-promoting and immunomodulatory impacts of commercial stimulants on Kuruma Shrimp, *Penaeus japonicus* (Bate, 1888) Juveniles, Egypt. J. Aquat. Biol. Fish., 25 (2021) 607–617.
[13] E.-S.H. Eissa, N.N.B. Abd El-Hamed, N.H. Ahmed, M.F. Badran, Improvement the hatchery seed production strategy on embryonic development and larval growth performance and development stages of Green Tiger Prawn, *Penaeus semisulcatus* using environmental aspects, Thalassas Int. J. Mar. Sci., 38 (2022) 1327–1338.
[14] E.-S.H. Eissa, N.H. Ahmed, A.A. El-Badawi, M.B. Munir, O.M. Abd Al-Kareem, M.E.H. Eissa, E.H.M. Hussien, S. El-Sayed Sakr, 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*, Aquacult. Res., 53 (2022) 6606–6615.
[15] M.T. El-Saadony, M.S. Ahmed, F.T. Taha, A.A. Najjar, N.M. Zabermawi, M.M. Nader, S.F. AbuQamar, K.A. El-Tarabily, A. Salama, Ali S. Selenium nanoparticles from *Lactobacillus paracasei* HM1 capable of antagonizing animal pathogenic fungi as a new source from human breast milk, Saudi J. Biol. Sci., 28 (2021) 6782–6794.
[16] S. Sehrawat, R. Khasa, A. Deb, S.K. Prajapat, S. Mallick, A. Basu, M. Surjit, M. Kalia, S. Vrati, Valosin-containing protein/p97 plays critical roles in the Japanese encephalitis virus life cycle, J. Virol., 95 (2021) e02336-20, doi: 10.1128/JVI.02336-20.
[17] K. Nalisa, S. Siripornadulsil, P. Sukon, W. Siripornadulsil, Antibacterial activity and genotypic–phenotypic characteristics of bacteriocin-producing *Bacillus subtilis* KKU213: potential as a probiotic strain, Microbiol. Res., 170 (2015) 36–50.
[18] M. Azimirad, S. Meshkini, N. Ahmadifard, S.H. Hoseinifar, The effects of feeding with synbiotic (*Pediococcus acidilactici* and fructooligosaccharide) enriched adult Artemia on skin mucus immune responses, stress resistance, intestinal microbiota and performance of angelfish (*Pterophyllum scalare*), Fish Shellfish Immunol., 54 (2016) 516–522.
[19] M.A.O. Dawood, S. Koshio, Application of fermentation strategy in aquafeed for sustainable aquaculture, Rev. Aquacult., 12 (2020) 987–1002.
[20] A. Neissi, G. Rafiee, M. Nematollahi, O. Safari, 2013. The effect of *Pediococcus acidilactici* bacteria used as probiotic supplement on the growth and non-specific immune responses of green terror, *Aequidens rivulatus*, Fish Shellfish Immunol., 35 (2013) 1976–1980.
[21] E. Ahmadifar, M.A.O. Dawood, M.S. Moghadam, A.H. Shahrestanaki, H.V. Doan, A.H. Saad, M. Aboubakr, E.Y. Abdelhiee, S.E. Fadl, The effect of *Pediococcus acidilactici* MA 18/5M on immune responses and mRNA levels of growth, antioxidant and immune-related genes in zebrafish (*Danio rerio*), Aquacult. Rep., 17 (2020) 100374, doi: 10.1016/j.aqrep.2020.100374.
[22] E. Ringø, S.H. Hoseinifar, K. Ghosh, H.V. Doan, B.R. Beck, S.K. Song, Lactic acid bacteria in finfish—an update, Front. Microbiol., 9 (2018) 1818, doi: 10.3389/fmicb.2018.01818.
[23] E. Ringø, H. Van Doan, S.H. Lee, M. Soltani, S.H. Hoseinifar, R. Harikrishnan, S.K. Song, Probiotics, lactic acid bacteria and bacilli: interesting supplementation for aquaculture, Special Issue: Fish and Shellfish Pathogens, J. Appl. Microbiol., 129 (2020) 116–136.
[24] M.A.O. Dawood, S. Koshio, M.M. Abdel-Daim, H. Van Doan, Probiotic application for sustainable aquaculture, Rev. Aquacult., 11 (2019) 907–924.
[25] M. Ghiasi, M. Binaii, A. Naghavi, H.K. Rostami, H. Nori, A. Amerizadeh, Inclusion of *Pediococcus acidilactici* as probiotic candidate in diets for beluga (*Huso huso*) modifies biochemical parameters and improves immune functions, Fish Physiol. Biochem., 44 (2018) 1099–1107.
[26] S. Torrecillas, F. Rivero-Ramírez, M.S. Izquierdo, M.J. Caballero, A. Makol, P. Suarez-Bregua, A. Fernández-Montero, J. Rotllant, D. Montero, Feeding European sea bass (*Dicentrarchus labrax*) juveniles with a functional synbiotic additive (mannan oligosaccharides and *Pediococcus acidilactici*): an effective tool to reduce low fishmeal and fish oil gut health effects?, Fish Shellfish Immunol., 81 (2018) 10–20.
[27] M.R. Rasha, M.S. Khaled, Evaluation of *Bacillus amyloliquefaciens* on the growth performance, intestinal morphology, hematology and body composition of Nile tilapia, *Oreochromis niloticus*, Aquacult. Int., 23 (2015) 203–217.
[28] S. Nath, C. Haldar, Effects of stress among shrimp post-larvae stocked at high stocking density in nursery culture system: a review, Int. J. Curr. Microbiol. Appl. Sci., 9 (2020) 2987–2996.
[29] Y. Gao, Z. He, H. Vector, B. Zhao, Z. Li, J. He, J.-Y. Lee, Z. Chu, Effect of stocking density on growth, oxidative stress and HSP 70 of pacific white shrimp *Litopenaeus vannamei*, Turk. J. Fish. Aquat. Sci., 17 (2017) 877–884.
[30] S.A. Kathyayani, M. Poornima, S. Sukumaran, A. Nagavel, M. Muralidhar, Effect of ammonia stress on immune variables of Pacific white shrimp *Penaeus vannamei* under varying levels of pH and susceptibility to white spot syndrome virus, Ecotoxicol. Environ. Saf., 184 (2019) 109626, doi: 10.1016/j.ecoenv.2019.109626.
[31] J. Yu, B.H. Koo, D.H. Kim, D.W. Kim, S.W. Park, *Aeromonas sobria* infection in farmed mud loach (*Misgurnus mizolepis*) in Korea, a bacteriological survey, Iran. J. Vet. Res., 16 (2015) 194–201.
[32] E.J. Noga, Fish Disease: Diagnosis and Treatment, 2nd ed., John Wiley and Sons, Hoboken, NJ, USA, 2010.
[33] H. Körkoca, Y. Alan, S. Bozari, M. Berktas, Y. Goz, Detection of putative virulence genes in Aeromonas isolates from humans and animals, J. Infect. Dev. Ctries, 8 (2014) 1398–1406.
[34] F.W.A. Sharifah, M.B. Munir, R. Asdari, Md.A. Hannan, Md.J. Hasan, Dietary lacto-sacc improved growth performance, food acceptability, body indices, and basic hematological parameters in empurau (*Tor tambroides*) fries reared in the aquaponics system, J. Appl. Aquacult., 35 (2023) 1131–1153.
[35] W. Horwitz, Official Methods of Analysis of AOAC International, Vol. I, Agricultural Chemicals, Contaminants, Drugs/Edited by William Horwitz, AOAC International, Gaithersburg (Maryland), 1997.

[36] E.G. Bligh, W.J. Dyer, A rapid method of total lipid extraction and purification, Can. J. Biochem. Physiol., 37 (1959) 911–917.
[37] AOAC, Official Methods of Analysis, 15th ed., Association of Official Analysis of Chemist, Washington, 2000.
[38] I. Adeshina, A. Jenyo-Oni, B.O. Emikpe, Use of *Eugenia cayrophyllata* oil as anaesthetic in farm raised African catfish *Clarias gariepinus* Juveniles, Egypt. J. Exp. Biol. (Zool.), 12 (2016) 71–76.
[39] K.M. Stoskopf, Fish Medicine, W.B. Saunders Company, Harcouttr Brace, Jovanovish Inc., 1993.
[40] P.C. Blaxhall, K.W. Daisley, Routine haematological methods for use with fish blood, J. Fish Biol., 5 (1973) 771–781.
[41] B.T. Doumas, H.G. Biggs, Determination of the Serum Globulin, Standard Methods of Clinical Chemistry, Academic Press, New York, 1972.
[42] T. Peters Jr., Serum albumin, Adv. Clin. Chem., 13 (1970) 37–111.
[43] T. Peters Jr., G.T. Biamonte, S.M. Durnan, In: Faulkner, W.P. Meites, Eds., Protein (Total Protein) in Serum, Urine and Cerebrospinal Fluid: Albumin in Serum, 9. American Association for Clinical Chemistry, Inc., Washington, D.C., 1982, pp. 317–325.
[44] S. Reitman, S.A. Frankel, A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases, Am. J. Clin. Pathol., 28 (1957) 56–63.
[45] D. Heinegård, G. Tiderström, Determination of serum creatinine by a direct colorimetric method, Clin. Chim. Acta, 43 (1973) 305–310.
[46] P. Trinder, Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor, Ann. Clin. Biochem.: Int. J. Lab. Med., 6 (1969) 24, doi: 10.1177/000456326900600108.
[47] B. Tengjaroenkul, B.J. Smith, T. Caceci, S.A. Smith, Distribution of intestinal enzyme activities along the intestinal tract of cultured Nile tilapia, *Oreochromis niloticus* L., Aquaculture, 182 (2000) 317–327.
[48] W. Schäperclaus, Fish Diseases, Vol. 1, CRC Press, Boca Raton, FL, 1992.
[49] S.R. Khalil, R.M. Reda, A. Awad, 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., 67 (2017) 119–128.
[50] R. Yossa, M. Verdegem, Misuse of multiple comparison tests and underuse of contrast procedures in aquaculture publications, Aquaculture, 437 (2015) 344–350.
[51] F.A. Salama, A. Abdel-Rahman, M. El Shehedy, Effect of adding *Pediococcus acidilactici* at low plant protein diets on growth performance of Nile tilapia (*Oreochromis niloticus*) fingerlings, Afr. J. Biol. Sci., 16 (2020) 93–105.
[52] R.R. Stickney, Principles of Warm Water Aquaculture, Wiley, New York, 1979.
[53] I.C. Zink, D.D. Benetti, P.A. Douillet, D. Margulies, V.P. Scholey, Improvement of water chemistry with *Bacillus* probiotics inclusion during simulated transport of Yellowfin Tuna Yolk Sac Larvae, North Am. J. Aquacult., 73 (2011) 42–48.
[54] C.E. Boyd, Water Quality: An Introduction, Springer Nature, 2019.
[55] M.A.E. Naiel, F.A. Mohamed, K.K. Doaa, S.A.A. Abd El-Hameed, E.M.G. Mansour, A.S.M. El-Nadi, A.A. Shoukry, Administration of some probiotic strains in the rearing water enhances the water quality, performance, body chemical analysis, antioxidant and immune responses of Nile tilapia, *Oreochromis niloticus*, Appl. Water Sci., 12 (2022) 209, doi: 10.1007/s13201-022-01733-0.
[56] 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, Aquacult. Res., 51 (2020) 1759–1770.
[57] E.M. John, K. Krishnapriya, T.V. Sankar, Treatment of ammonia and nitrite in aquaculture wastewater by an assembled bacterial consortium, Aquaculture, 526 (2020) 735390, doi: 10.1016/j.aquaculture.2020.735390.
[58] T. Liu, X. He, G. Jia, J. Xu, X. Quan, S. You, Simultaneous nitrification and denitrification process using novel surface-modified suspended carriers for the treatment of real domestic wastewater, Chemosphere, 247 (2020) 125831, doi: 10.1016/j.chemosphere.2020.125831.
[59] P.R. Rout, P. Bhunia, R.R. Dash, Simultaneous removal of nitrogen and phosphorous from domestic wastewater using *Bacillus cereus* GS-5 strain exhibiting heterotrophic nitrification, aerobic denitrification and denitrifying phosphorous removal, Bioresour. Technol., 244 (2017) 484–495.
[60] J. Yousuf, J. Thajudeen, M. Rahiman, S. Krishnankutty, A.P. Alikunj, M.H.A. Abdulla, Nitrogen fixing potential of various heterotrophic *Bacillus* strains from a tropical estuary and adjacent coastal regions, J. Basic Microbiol., 57 (2017) 922–932.
[61] Y. Deng, M.C.J. Verdegem, Ep Eding, F. Kokou, Effect of rearing systems and dietary probiotic supplementation on the growth and gut microbiota of Nile tilapia (*Oreochromis niloticus*) larvae, Aquaculture, 546 (2022) 737297, doi: 10.1016/j.aquaculture.2021.737297.
[62] E.R. El-Haroun, A.M.A-S. Goda, M.A.K. Chowdhury, Effect of dietary probiotic Biogen supplementation as a growth promoter on growth performance and feed utilization of Nile tilapia *Oreochromis niloticus* (L.), Aquacult. Res., 37 (2006) 1473–1480.
[63] H. Van Doan, E. Wangkahart, W. Thaimuangphol, P. Panase, N. Sutthi, Effects of *Bacillus* spp. mixture on growth, immune responses, expression of immune-related genes, and resistance of Nile tilapia against *Streptococcus agalactiae* infection, Probiotics Antimicrob. Proteins, 15 (2023) 363–378.
[64] R. De Schrijver, F. Ollevier, Protein digestion in juvenile turbot (*Scophthalmus maximus*) and effects of dietary administration of *Vibrio proteolyticus*, Aquaculture, 186 (2000) 107–116.
[65] I.G. de la Banda, C. Lobo, M. Chabrillón, J.M. León-Rubio, S. Arijo, G. Pazos, L.M. Lucas, M.Á. Moriñigo, Influence of dietary administration of a probiotic strain *Shewanella putrefaciens* on senegalese sole (*Solea senegalensis*, Kaup 1858) growth, body composition and resistance to *Photobacterium damselae* subsp *piscicida*, Aquacult. Res., 43 (2012) 662–669.
[66] D.-A. Schütt, J. Lehmann, R. Goerlich, R. Hamers, Haematology of swordtail, *Xiphophorus helleri*. I: blood parameters and light microscopy of blood cells, J. Appl. Ichthyol., 13 (1997) 83–89.
[67] S.M. Aly, Y.A.-G. Ahmed, A.A.-A. Ghareeb, M.F. Mohamed, Studies on *Bacillus subtilis* and *Lactobacillus acidophilus*, as potential probiotics, on the immune response and resistance of Tilapia nilotica (*Oreochromis niloticus*) to challenge infections, Fish Shellfish Immunol., 25 (2008) 128–136.
[68] E. Capkin, I. Altinok, Effects of dietary probiotic supplementations on prevention/treatment of yersiniosis disease, J. Appl. Microbiol., 106 (2009) 1147–1153.
[69] S.A. Abdelnour, S. Ghazanfar, M. Abdel-Hamid, H.M.R. Abdel-Latif, Z. Zhang, M.A.E. Naiel, Therapeutic uses and applications of bovine lactoferrin in aquatic animal medicine: an overview, Vet. Res. Commun., 47 (2023) 1015–1029.
[70] B. Magnadottir, Immunological control of fish diseases, Mar. Biotechnol., 12 (2010) 361–379.
[71] F. Genten, E. Terwinghe, A. Danguy, Atlas of Fish Histology, Science Publishers, Enfield, NH, USA, An Imprint of Edenbridge Ltd., 2009.
[72] J.M. Bates, E. Mittge, J. Kuhlman, K.N. Baden, S.E. Cheesman, K. Guillemin, Distinct signals from the microbiota promote different aspects of zebrafish gut differentiation, Dev. Biol., 297 (2006) 374–386.
[73] D.L. Merrifield, G. Bradley, R.T.M. Baker, S.J. Davies, Probiotic applications for rainbow trout (*Oncorhynchus mykiss* Walbaum) II. effects on growth performance, feed utilization, intestinal microbiota and related health criteria postantibiotic treatment, Aquacult. Nutr., 16 (2010) 496–503.
[74] P. Li, Q. Wen, D.M. Gatlin III, Dose-dependent influences of dietary β-1,3-glucan on innate immunity and disease resistance of hybrid striped bass *Morone chrysops*×*Morone saxatilis*, Aquacult. Res., 40 (2009) 1578–1584.
[75] 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 (2019) 1367–1377.

[76] A.A. Al-Hamidi, Properties and applications of proteinaceous antibiotics produced by lactic acid bacteria: a review, J. Saudi Chem. Soc., 8 (2004) 35–46.

[77] S.H. Hoseinifar, Y.-Z. Sun, A. Wang, Z. Zhou, Probiotics as means of diseases control in aquaculture, a review of current knowledge and future perspectives, Front. Microbiol., 9 (2018) 2429, doi: 10.3389/fmicb.2018.02429.

[78] S. Lee, K. Katya, A. Hamidoghli, J. Hong, D.-J. Kim, S.C. Bai, Synergistic effects of dietary supplementation of *Bacillus subtilis* WB60 and mannanoligosaccharide (MOS) on growth performance, immunity and disease resistance in Japanese eel, *Anguilla japonica*, Fish Shellfish Immunol., 83 (2018) 283–291.