Saudi Journal of Biological Sciences 30 (2023) 103539

HOSTED BY

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Growth promoter, immune response, and histopathological change of prebiotic, probiotic and synbiotic bacteria on Nile tilapia

F.M Ghaly [a], Shahira H.M Hussein [b], Somayah M. Awad [c], Abeer A. EL-Makhzangy [a,*]

[a] Department of Botany and Microbiology, Faculty of Sciences, Zagazig University, Sharkia Governorate, Zagazig, Egypt
[b] Chief Research of Pharmacology, Pharmacology Department, Animal Health Research Institute, Zagazig Branch, Agriculture Research Center, Sharkia Governorate, Zagazig, Egypt
[c] Chief Research of Fish health and Management Department, Central Laboratory for Aquaculture Research, Abassa, Abu-Hammad, Sharkia, Agriculture Research Center, Sharkia Governorate, Zagazig Egypt

ARTICLE INFO

Article history:
Received 15 July 2022
Revised 10 November 2022
Accepted 7 December 2022
Available online 13 December 2022

Keywords:
Probiotic
Prebiotic
Synbiotic
V.anguillarium

ABSTRACT

This study aimed at determining the influence of prebiotic, probiotic, and synbiotic supplemented diets on *Oreochromis niloticus*. Fish with initial body weight (25.8 ± 1.2) g and length range from (13.5 ± 1.5) cm were collected and randomized to four dietary treatments for 60 days. Furthermore, fish were divided into three groups in triplicate; A0 control (-ve), A1 control (+ve) infected with *V.anguillarium,* and a third non-treated group. Moreover, the third group further separated into two groups, A and B. Group (A) was treated with prebiotic, probiotic, and symbiotic (A2, A3, and A4), while group (B) was infected with *V.anguillarium* then treated with prebiotic, probiotic and symbiotic (A5, A6, and A7). The results revealed that all treatments supplemented with synbiotics represented highly significant increase ($p \leq 0.05$) in (SGR), BWG percentage, relative growth rate (%), lysozyme activity, IMG, SOD, and CAT. At the same time, they exhibited a significant decrease in MAD and FCR. Besides, fish that feed dietary supplementation with prebiotics, probiotics, and synbiotics revealed a significant increase in RBCs, WBCs, and Hb. In contrast, they showed a significant decrease in ALT, AST, albumin, total protein, globulin, creatinine, and urea compared with control. Additionally, high survival rates were recorded in groups that received a diet supplemented with probiotics, followed by prebiotics and synbiotics.

© 2022 The Authors. Published by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Aquaculture has grown faster than capture fisheries in the past two years and is expected to expand further over the next decade. Fish represent over 20 % of animal protein intake in twenty African countries (FAO, 2017). However, in some cases, decreasing in quality of water, increasing in stress, or lower food quality may lead to presence of new pathogens and infectious diseases that caused by bacteria, viruses, and parasites causing significant problems in the fish farming industry (Geng et al. 2012; Abumourad et al. 2013). Asia in 2012 recorded up to 3.3 million tonnes of the production of tilapia in the aquaculture sector (FAO, 2014). Bacterial disease in the aquaculture production system is caused by some causative agents such as *Enterobacteriaceae*, *Pseudomonadaceae,* or *Vibrionaceae*, these three families have short gram-negative rods. In contrast, long rod gram-negative bacteria, *Flavobacterium psychrophilum* and *Flavobacterium columnare* of the family Flavobacteriaceae may cause heavy mortality in fish stock (Aly, 2009; Barbosa et al. 2011; McBride,2014). Vibriosis disease reported in fresh and marine water causes significant mortality (7.50 %) in fish culture. *Vibrio* infections can spread rapidly in heavily stocked. Seven *Vibrio* species have been associated with a disease in the fish, identified as *Vibrio anguillarum, V. ordalli, V. vulnificus, V. carchariae, V. damsel,* and *V. alginolyticus* (Reed & Franeis-Floyd 1996). The usual strategy for controlling fish disease, which was used over a long time, was the administration of antibiotics before dealing with a bacterial

Abbreviations: GC-MS, Gas chromatography–mass spectrometryanalysis; MRS, De Man, Rogosa and Sharpe medium; TCBS, Thio sulfate citrate bile salt sucrose agar; TSA, Tryptic Soya Agar; TSB, Tryptic Soya broth; R-S, Rimler – Shotts; cfs, cell-free supernatant; IGM, immunoglobin M.

* Corresponding author at: Department of botany and Microbiology, faculty of science, Zagazig university, Sharkia Governorate, Zagazig, Egypt.

E-mail addresses: m.elghaly@zu.edu.eg (F.M Ghaly), Drshahirahanafy@ahri.gov.eg (S.H.M Hussein), dr.somayahawad.clar@arc.sci.eg (S.M. Awad), a.ibrahim22@science.zu.edu.eg (A.A. EL-Makhzangy).

Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2022.103539
1319-562X/© 2022 The Authors. Published by Elsevier B.V. on behalf of King Saud University.
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

infection. However, the widespread of using antibiotics in aquaculture faces severe problems due to its various harmful effects by accumulation in tissue, development of antibiotic-resistant bacteria, and immunosuppression, and therefore devastation the bacterial environment and finally might cause water pollution (Alcaide et al. 2005; Yousefian and Amiri, 2009).

Probiotics play an essential role as alternatives to chemotherapy and treatments with antibiotics (Rekiel et al. 2007). They administer more useful probiotics through routine water and nutritional supplements either alone or added with other probiotics, prebiotics, and other immunostimulants (Hai & Fotedar 2009). Common probiotics used widely in the aquaculture system belongs to *Saccharomyces, Lactobacillus, Clostridium, Bacillus* and *Shewanella* (Kim et al. 2017).

The application of probiotics as an evirnomentally friendly alternative to antibiotics is an emerging strategy for sustainable aquaculture. (Silva et al. 2013; Tan et al. 2019). *Bacillus. subtilis, B. amyloliquefaciens* and *B. cereus* were Gram-positive bacteria and have inhibiting effect against Gram-negative bacteria, heat stability, the ability to tolerate a high proportion of bile, spore formation, acidity, also capable to produce digestive enzymes and colonizing in gut (Hong et al. 2005; Reda et al. 2018).

Prebiotics known as un-digested food components so as to stimulate the activity of beneficial intestinal bacteria and their growth as probiotic bacteria in the host, therefore improving the health of host (Guerreiro et al. 2018). The combination prebiotics and probiotics in a synergistic form to enhance probiotics' beneficial effects can be defined as synbiotics (Cerezuela et al. 2011). Synbiotics promise improvement various health benefits such as resistance to bacterial infection in the gut, antibacterial effect, and immune-modulatory activities (Kamoura et al., 2020).

1.1. Objective of the research

i) The aim of the present study is to investigate the effects of supplementation of a probiotic (*Bacillus amyloliquefaciens* SW19), the prebiotic and their synbiotic interaction on growth performance, hematological, biochemical blood parameters and histopathological changes of the Nile tilapia (*Oreochromis niloticus*).
ii) The study evaluates that prebiotic, probiotic, and synbiotic bacteria can be used as a safe alternative strategy for fish disease management to antibiotics.

2. Material and methods

2.1. Collection of the samples

Collecting naturally infected Nile tilapia from private farms at sun El-Hagar, Kanater, Kalubia Governorate, and El Behira from October 2017 to August 2019. All infected fish were examined for pathogenic bacteria.

2.2. Isolation and identification of pathogenic bacteria

Isolatation of bacteria from internal organs (spleen, liver and kidney) and muscles on TSB broth, incubated for 18 h at 28c, then a loop from broth culture was taken and streaked onto TCBS, R-S medium, cetrimide for *Vibrio*, *Aeromonas*, and *Pseudomonas* species isolation. The selected pure colony was restreaked on (TSA) media, incubated for 24–48 h at 37 °C for morphological and biochemical tests according to (Austin and Austin, 2007).

2.3. The inhibitory activity of probiotic bacteria in-vitro

Four probiotic bacteria were used; the first one was kindly provided from the midgut of larva, determined by Gene bank accession No MK160141, *B. amyloliquefacien* SW19 (Shaimaa, 2019). The second was a commercial Baker's yeast *Saccharomyces cerevisiae*. The other two were isolated from pickles and yogurt identified morphologically and biochemically as *Bacillus cereus* and *Lactobacillus bulgaricus,* respectively. These probiotics exhibited *in-vitro* antagonistic activity against previously isolated pathogenic bacteria, such as *Aeromonas hydrophila, A.jandiae,* and *A.caviae, Pseudomonas aeruginosa, Vibrio anguillarum* and *V. alginolyticus* by the disc–agar diffusion technique. Applying sterile cotton swabs, the probiotic cultures were supplemented in sterilzed nutritional broth for 6–8 h at 37 °C. Using flame-covered ethanol forceps and, cultures were aseptically wiped onto the surface of sterile Mueller-Hinton agar plates. Bacteria previously isolated discs placed overseeded sufficiently separated from each other to avoid overlapping inhibition zones, and kept at 37 °C for 24 h. Measuring diameter of inhibition zones in mm (Muniruzzaman and Chowdhury, 2004).

2.4. Identification the metabolites of Bacillus amyloliquefaciens SW19 by GC/MS

The GC–MS system (Agilent Technologies) was equipped with gas chromatograph (7890B) and mass spectrometer detector (5977A) at Central Laboratories Network, National Research Centre, Cairo, Egypt. The GC was equipped with HP-5MS column (30 m × 0.25 mm internal diameter and 0.25 μm film thickness). Analyses were carried out using hydrogen as the carrier gas at a flow rate of 1 ml/min at a splitless, injection volume of 1 μl and the following temperature program: 50 °C for 1 min; rising at 5 °C /min to 100 °C and held for 0 min; rising at 10 °C/min to 300 °C and held for 5 min. The injector and detector were held at 250 °C and 260 °C, respectively. Mass spectra were obtained by electron ionization (EI) at 70 eV and using a spectral range of *m*/*z* 50–550 and solvent delay 6 min. Identification of different constituents was determined by comparing the spectrum fragmentation pattern with those stored in Wiley and NIST Mass Spectral Library data.

3. Preparation of fish diet

3.1. Probiotics bacteria supplemented diet preparation

According to an *in-vitro* study, *Bacillus amyloliquefaciens* SW19 has high antibacterial activity against pathogens. One ml of 24 h old culture was centrifuged at 3000 rpm untile 30 min at 4C, the pellets were harvested and washed twice with sterile saline, subsequently re-centrifuged again at 3000 rpm for 5 min, and the final concentration has been adjusted to 10^{10} CFU/ml in saline using a McFarland standard tube (Selim et al. 2019), then added 50 ml/kg saline containing probiotic bacteria to the basal diet (Salam et al. 2021) to give an initial number of 10^{10} CFU/ml diet and dried at room temperature under aseptic conditions for 6 h, and preserved at 4 °C (Aly et al. 2008).

3.2. Prebiotics bacteria supplemented diet preparation

In 500 ml of MRS broth medium Probiotics were inoculated then incubated for 4 days at 37 °C. After incubation, CFS obtained by centrifuging cultured bacteria in 4000 xg for 15 min, then filtrated the supernatant via syringe filters (0.45) μm pore size (Akabanda et al. 2014). Then, ethyl acetate solvent was added to

filtrate in the ratio 1:1 v/v, sonicated for 20 min. separated the mixture three times within funnel of separating, evaporated the crude extract to reach tenfold in concentration. The bioactive metabolites were extracted according to (Liu et al. 2008; Shaimaa,2019). Then, the pure extract was added to the basal diet at 2 g/kg (Grisdale–Helland et al. 2008).

3.3. Synbiotic bacteria supplemented diet preparation

The synbiotic culture of *Bacillus amyloliquefacien* was mixed with *Bacillus amyloliquefacien* extract and after mixing with the feed with 0.4 g/kg according to (Jahari et al. 2018) (Table S1).

3.4. Fish and experimental design

Apparently, two hundred and forty healthy (*Oreochromis niloticus*), the average body weight (25.9 ± 1.2 g) were collected from the control laboratory for aquaculture, Abassa fish farm in Sharkia province, Egypt. They were allocated randomly to equally-three groups. Every group had three replicates distributed as ten fish per aquarium. The first group, A0 control(-ve), received a basal diet free from any additives. The second group, A1 control(+ve), was infected with *V. anguillarium,* previously isolated from naturally infected Nile tilapia and non-treated also fed basal diet, divided the third group into two subgroups; A & B. Group (A) was further divided into three subgroups (A3, A4, and A5) treated immediately by dietary supplemented with probiotics (*B. amyloliquefacien* 1×10^{10} CFU/g), prebiotics (*B.amyloliquefacien*- ethyl acetate extract)and synbiotics (probiotic + prebiotic) respectively. Also, group (B) was divided into three subgroups (A5, A6, and A7) infected with *V. anguillarium* then treated through feeding dietary supplemented with probiotics, prebiotics, and synbiotics, respectively. For 8 weeks fish were tested fed-diet at the rate of 3 % of live body weight three times daily (Silva et al. 2015). Aquaria were provided with tap water de-chlorinated, and average temperature 25℃ ± 1. Also, oxygen was adjusted for continuous aeration using electrical air pumping compressors. Fish per aquarium were weighted weekly from the beginning of the feeding experiment, and the diet amount was adjusted.

3.5. Challenging fish with pathogen

After two weeks from begging of the experiment, four groups (A1, A5, A6, and A7) were injected I/P by 0.1 ml of saline including 10^8 CFU ml^{-1} of *V. anguillarum* per fish, the strain previously isolated from naturally infected fish, while A0 I/P was injected by 0.1 ml sterile saline as control negative (Yu et al. 2019). A1 acts as a control positive infected and non–treated, while A5, A6, and A7 were infected and treated immediately with probiotics, prebiotics, and synbiotics, respectively (Al-Dohail et al. 2008). At the final of the experimence, A2, A3, also A4 were inoculated with 0.1 ml of pathogenic bacteria *V. anguillarium*. All fish injected were observed for any abnormalities signs and mortality for 14 days daily. Any dead fish were recorded immediately for post-mortem examination and routine histopathological examination.

3.6. Parameters of feed utilization and growth performance

Calculation of (SGR), (FCR), (WG) accordingly to the following equations.

3.6.1. Specific growth rate (SGR)

SGR% =100(ln final body weight–ln initial body weight/ experimental period (day) (Siddiqui et al. 1988).

3.6.2. Feed conversion ratio (FCR)

FCR%=feed intake (g)/body weight gain (g) × 100 (Desilva and Anderson, (1995); Yanbo and Zirong, 2006).

3.6.3. Average weight gain (AWG)

WG% (g/fish) = (average final weight(g)–average initial weight (g) /average initial weight (g) × 100 (Jauncey and Ross, 1982).

3.7. Blood sampling

3.7.1. The collection of blood samples

Two samples of blood were withdrawn from the caudal blood vessels then collected on seventh-day post-challenge and at the end of the experiment to evaluate some immunological parameters and assess some biochemical parameters. The first were taken with EDTA and the second free of EDTA, centrifuged in 3000 rpm till 15 min for separation of serum. The determination of immunoglobulin (IgM) using a kit of ELISA, serum germicidal activities, and serum lysozyme against *Vibrio* spp. determined suspension by the method of (Kajita et al., 1990; Rajaraman et al. 1998).

3.7.2. Non-specific immune parameters

Blood was collected from fish in the caudal vein with puncture to determine lysozyme activity. After that, allowed to coagulate for 30 min at room temperature. Removed Serum from the coagulated sample after centrifugation in 3000 rpm for 5 min then frozen at −20 °C for chemical analysis, according to Parry et al. (1965). IGM was measured by an ELISA according to (Fuda et al. 1991).

3.7.3. Tests of liver functions

Liver enzymes such as (ALT) alanine aminotransferase and (AST) aspartate aminotransferase activities were evaluated according to Reitman and Frankel (1957). Assayed the plasma within 24 h next thawed and stored on ice. The frozen liver sample was stored at −80 °C, centrifugation at 3000 rpm for 10 min at 4 °C. collected the supernatants cautiously. Then, kept for enzyme analysis at −80 °C, immune-related enzymes such as myeloperoxidase (MPO), and the relevant- indicators of antioxidant in plasma, including liver superoxide dismutase (SOD), malondialdehyde (MDA) (Quade & Roth, 1997).

3.8. Studies of histopathological changes

Samples from the gills, kidney, and liver were taken before and post-treatment, then fixed in 10 % buffered formalin for histopathology (Bancroft and Stevens, 1996).

3.9. Statistic

ANOVA one-way used for analyzed all data. Differences among means were tested through Duncan´s multi-range test. The results were presented as average values with standard errors (mean ± SE) by using the SPSS 1600 statistical package (SPSS Inc., 1997, **USA**).

4. Results

4.1. Isolation and identification of pathogenic bacteria

All isolates were identified phenotypically according to standard protocol described bacterial isolation and identification. The major isolates were recorded as Gram-negative bacteria *Aeromonas sobria, A.jandiae, A.caviae, A.veronii,* and *A. hydrophila, Pseudomonas* spp. *(P. aeroginosa&P.medocina), and Vibrio* spp. *(V. anguillarum, V. alginolyticus, and V. paraneamolyticus*),while the minor ratio were

recorded as Gram-positive bacteria *Staphylococcus spp* and *Bacillus spp* the trace ratio.

4.2. The inhibitory activity of probiotic bacteria in-vitro

As shown in Fig. 1 and Table 1, *B. amyloliquefacien* SW19 was the most active antagonism against pathogens in which high inhibition zone was recorded. It had an antagonistic effect against isolated pathogenic bacteria (*A. caviae*, *A. hydrophila*, *A. jandiae*, *P. aeroginosa*, *V. anguillarum,* and *V. alginolyticus*). *V. anguillarum* was more sensitive to probiotics bacteria with a 40 mm diameter, while *A. caviae* and *P. aeroginosa* recorded the lowest inhibition zone.

4.3. Chemical classification of ethyl acetate extract by GC/MS chromatography

The chemical constituents of metabolites extracted from *Bacillus amyloliquefaciens* SW19 (CFS) were analyzed by GC/MS. Generally, the ethyl acetate extract of CFS contained 6 compounds represented in Table 2, (Fig. 2 & Fig. 3). The high value was recorded at Citroflex A, followed by bis (2-ethylhexyl) phthalate, then butyl citrate, 1-Propene-1,2,3-tricarboxylic acid, tributyl ester and 5-(Prop-2-enoyloxy) pentadecane. The lowest ratio was 3-(prop-2-enoyloxy) dodecane. The previous compound had antibacterial activity against *Aeromonas* spp., *Pseudomonas* spp., and *Vibrio spp*.

4.4. Challenging fish with pathogen

From (Table S2), it was noticed that the high mortality percentage in A1 (infected with *V. anguillarium* and non-treated) as 90 %. In contrast, low mortality was observed in groups A2, A3, and A4, which were directly fed dietary supplements with probiotics, prebiotics, and synbiotics (6.7, 16.7, and 23.3), respectively, compared with control group A0 (46.67 %). Also, a high survival rate was reported in A2 (93.33). After 8th weeks, no mortality was recorded in groups (A2, A3, and A4) that were fed probiotics, prebiotics, and synbiotics then infected with *V. anguillarium*.

4.5. Growth performance

From Table 3, it was observed that fish in A2, A3, A4, A5, A6, and A7 significantly increased in relative growth rate, final body weight, specific growth rate, and body gain, the high valuable, represented in A4 and A7 as (53.20, 52.23), (26.09, 24.97), (95.92, 91.57 %), and (1.16, 1.08), respectively. In the opposite direction, these groups showed a significant decrease in feed conversion ratio in comparison to control and others treatment groups. lowest final weight were represented in the control diet.

4.6. Hematological and biochemical parameters

4.6.1. Hematological parameters

In the first week, as represented in Table S3, groups which received diets supplemented with prebiotics, probiotics, and synbiotics were different *($p \leq 0.05$)* from the control in hemoglobin density, RBCs, also WBCs. In the case of RBCs, high values were recorded in A2, A3, and A5. The lowest values were represented in the A1 group (infected non-treated). However, A1 recorded a highly significant increase in WBCs compared with control A0. Also, the highest hemoglobin density values were observed in groups that received a diet supplemented with synbiotics (A4 and A7), followed by (A2, A3, and A6). The lowest value was shown in A1, compared with control A0. Decreasing WBCs value were occured in groups that fed diet supplemented with probiotics, prebiotics, and synbiotics, to reach control group A0, in the final of the experiment but stile high in A1, as presented in (Table S4).

4.6.2. Immune parameters

After 60 days, lysozyme activity, IgM (immunoglobin), SOD (superoxide dismutase), and CAT (catalase) showed a significant increase in groups A2, A3, A4, G5, A6, and A7. The lowest values were obtained in A1 compared to control, as seen in (Table S5).

All fish in A2, A3, A4, A5, A6, and A7 groups displayed significantly reduced plasma MAD (malondialdehyde) levels; the opposite in A1 showed a significant increase compared with A0. As shown in Table S6, after 7 days of the experiment highly significant decrease was observed in ALT (Alanine aminotransferase), total protein, albumin, and globulin levels in groups A2, A3, A4, A5, A6, and A7; while a significant increase in AST (Aspartase aminotransferase) were recorded in the same groups. Also, both ALT and AST recorded a high significantly increase at A1, but albumin, globulin, and total protein indicated a significant reduction compared to control.

The levels of creatinine and urea showed a significant decrease in groups A2, A3, A4, A5, A6, and A7; they also revealed high rising in A1 comparison with control A0. The A/G ratio showed a significant increase in A3, A4, A6, and A7 groups but showed a significant decrease in A2, A5, and A1 groups compared with the control group.

After 60 days of the experiment, as in Table S7, the level of ALT was reduced in groups A2, A3, A5, and A6, while in groups A4 and A7 showed a significant increase with a highly significant increase in A1. AST, represented a significant decrease in A4; conversely, groups A2, A3, A5, A6, and A7 was increased. Also high value was obtained at group A1 in compared with A0. Total protein, albumin and globulin showed significant increase in groups A2, A3, A4, A5, A6, and A7 and a highly significant decrease in A1 compared with A0. The A/G ratio revealed a significant increase in A4, A5, A6, and A7, and a significant decrease in A2 and A3 groups compared to control. The urea and creatinine levels showed a significant

Fig. 1. Antagonistic activities of probiotic bacteria with pathogenic bacteria.No(1):*Bacillus cereus*, (2):*Saccharomyces cerevisiae*, (3):*Lactobacillus bulgaricus,* and (4):*Bacillus amyloliquefaciens* SW19

Table 1
The antagonistic effect of probiotics bacteria against isolated pathogenic bacteria.

Pathogenic bacteria	Diameter of inhibition zones (mm)			
	Bacillus amyloliquefaciens SW19	*Bacillus cereus*	*Lactobacillus bulgaricus*	*Saccharomyces cerevisiae*
A.jandiae	25	13	20	18
A.caviae	15	15	–	10
A. hydrophila	20	16	20	15
V.anguillarum	40	15	20	20
V. alginolyticus,	35	–	15	15
P.aeroginosa	20	10	–	16

*Antibacterial activity against (A): V.anguillarum**;** (B): A.caviae&(C): A. hydrophila.

Table 2
GC/MS chromatogram for the extraction of *Bacillus amyloliquefaciens.*

Peak	RT	The name	Formula	The area	Total area %
1	26.995	1-Propene-1,2,3-tricarboxylic acid, tributyl ester	C18H30O6	262345.31	0.52
2	27.25	Butyl citrate	C18H32O7	526250.48	1.04
3	28.277	Citroflex A	C20H34O8	43,644,639	86.1
4	28.763	3-(Prop-2-enoyloxy)dodecane	C15H28O2	219757.78	0.43
5	30.223	Bis(2-ethylhexyl) phthalate	C24H38O4	5814046.3	11.47
6	31.761	5-(Prop-2-enoyloxy)pentadecane	C18H34O2	224614.04	0.44

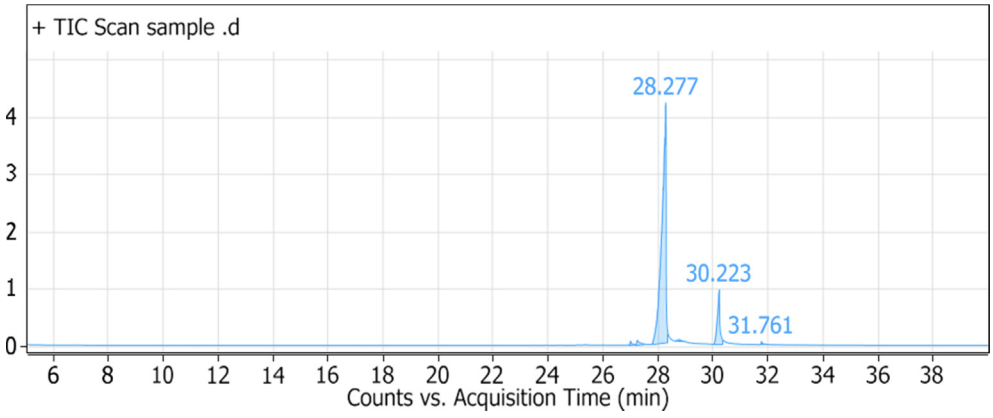

Fig. 2. GC/MS of *Bacillus amyloliquefaciens* SW19 metabolites extract.

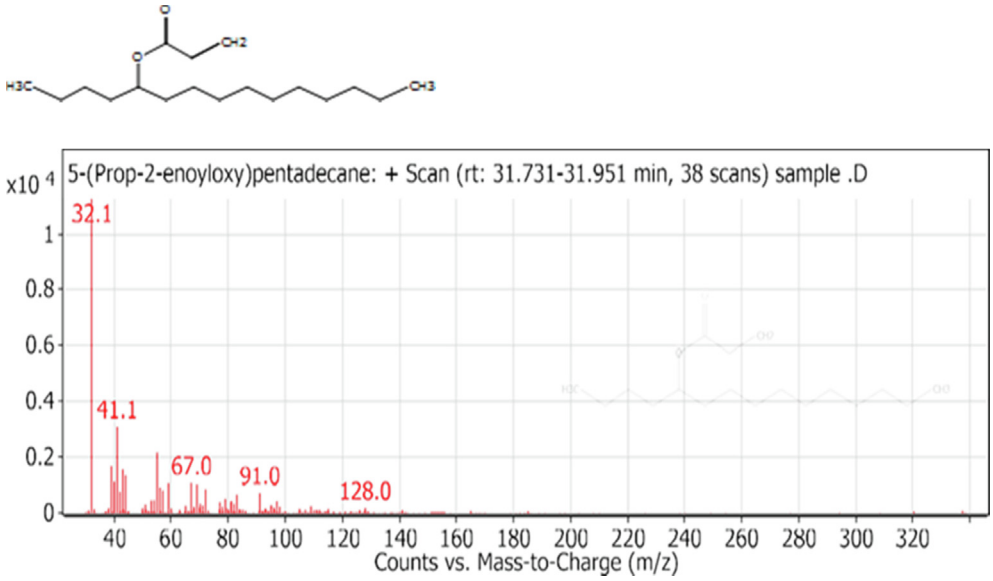

Fig. 3. GC/MS fraction of antibacterial substance (5-(prop-2-enoyloxy) pentadecane) produced from *Bacillus amyloliquefaciens* SW19.

Table 3
Influence of probiotics, prebiotics & synbiotics supplemented in the diet for 8 weeks on growth performance parameters of *O. niloticus* (M ± S.E).

Parameters	Experimental Fish						
	A0	A2	A3	A4	A5	A6	A7
Initial BW (g)	27.17 ± 3.28 ab	26.80 ± 2.65 ab	26.57 ± 2.72 ab	27.20 ± 1.73 ab	26.90 ± 2.31 ab	26.53 ± 3.28 ab	27.27 ± 2.60 a
Final BW (g)	32.32 ± 5.01 d	47.50 ± 3.79b	37.08 ± 4.33c	53.20 ± 3.11 a	39.32 ± 2.52 bc	38.73 ± 4.41 bc	52.23 ± 34.93 ab
Body gain (g)	5.15 ± 3.69 d	20.70 ± 1.53 ab	10.52 ± 6.17c	26.09 ± 1.44 a	12.42 ± 1.33b	12.20 ± 1.15b	24.97 ± 34.67 a
Relative growth rate (%)	18.99 %	77.24 %	39.59 %	95.92 %	46.17 %	45.99 %	91.57 %
SGR (%)	0.29 ± 0.35 d	0.95 ± 0.32 ab	0.56 ± 0.35c	1.16 ± 0.38 a	0.63 ± 0.08b	0.63 ± 0.62b	1.08 ± 0.15 a
FCR	9.58 ± 0.08 a	3.33 ± 0.07 cd	4.55 ± 0.26b	1.87 ± 0.51 d	3.89 ± 0.07 bc	3.92 ± 0.23 bc	2.03 ± 0.31 cd

A0: apparently healthy fish without any treatment (control group). A2: apparently healthy fish, fed basal diet + probiotic. A3: apparently healthy fish fed basal diet + prebiotic. A4: apparently healthy fish, fed basal diet + symbiotic. A5: infected fish and treatment with probiotics. A6: infected fish then treated with prebiotics. A7: infected fish and treated with synbiotics. Within the same columns mean values with various superscript letters were significantly differed at ($p \leq 0.05$). SGR:(Specific growth rate), FCR:(Feed conversion ratio), BW:(body weight).

decrease in A2, A3, and A4 groups while groups A5, A6, A7, and A1 were showed high result, in compared with control.

4.7. Histopathological changes

The results revealed certain changes affecting the parenchymatous organs (liver and kidney) after 60 days of dietary supplementation with probiotics, prebiotics, and synbiotics. All examined organs were normal and with no evidence of any abnormalities in the A0 control group as in (Fig. 4). Some histopathological changes occurred in the kidney and liver of group A1. The kidney in microscopic examination showed degeneration from some renal tubules. The renal blood vessels diffuse severe congestion while the liver showed hyperplasia of hematopoietic tissue invested large blood vessels and diffuse vacuolation of hepatocytes, as represented in (Fig. 5).

In groups A2, A3, and A4, liver tissue had less liver congestion degree than without supplementation, as shown in Fig. 6 and (figS1&S2). The kidney showed mild degenerative changes of renal tubules and renal blood vessels congestion with apparently normal renal cortex.

The liver of Nile tilapia in group A5 showed mild congestion of hepatic blood vessels with hyperplasia of hematopoietic tissue invests, kidney showed mild activation of the melanomacrophage center with apparently normal renal cortex, as shown in (Fig S3). The focal area of extravasated erythrocytes with focal vacuolation was represented in the liver of group A6. It was noticed that the kidney showed severe diffuse congestion of renal blood vessels in addition to mild focal degeneration of renal tubules with focal vacuolation, as shown in (Fig S4).

In the last group, A7, the liver showed perivascular leucocytic cells infiltration with congestion of hepatic blood vessels. Also, focal leucocytic cells infiltrating the renal cortex were shown in the kidney, as represented in (Fig S5).

5. Discussion

Recently, probiotics and prebiotics became an integral part of aquaculture practices that improve disease resistance and growth performance **(**Mehrabi et al. 2012**)**. In principle, probiotics and prebiotics have been combined to provide the probiont with a advantage of competitive over competing domestic groups, thus improving implantation and the survival of lived microbial nutritional supplements at the digestive tract of the host **(**Gibson and Robefroid, 1995).

The study demonstrated that *Bacillus amylolqufiense SW19* had an antagonistic effect against isolated pathogenic bacteria; *Aeromonas caviae, A hydrophila.*,and *A. jandiae, Pseudomonas* spp. *(P. aeroginosa),* and *Vibrio* spp. *(V. anguillarum and V. alginolyticus.)*. Our results agree with those of **(**Krishnan, 2014**),** who noticed that *Bacillus* spp. had a probiotic effect *in-vitro* against *A. hydrophila* and *V. harveyi* by producing bacteriocin–like substance. Antibacterial effect of *Bacillus* species may be attributed to variable causes, included pH change of bacterial growth media, production of polypeptides and volatile compounds, and nutrient competition **(**Balcázar and Rojas-Luna (2007). In the present investigation, six compounds were obtained from extraction of *B. amyloliquefaciens SW19* ethyl acetate, (1-Propene-1,2,3-tricarboxylic acid, tributyl ester, Butyl citrate, Citroflex A, 3-(Prop-2-enoyloxy) dodecane, Bis (2-ethylhexyl) phthalate and 5-(Prop-2-enoyloxy) pentadecane), the analysis was done by using GC–MS. Our result coincides with those obtained by Gadhi et al., (2018); Sivarajan et al., (2019)**;** and Masika et al., (2020) who demonstrated that the use of GC–MS analysis for detecting the compounds biodegradable in waste petroleum hydrocarbon using *Bacillus* spp consortia. The antibacterial activities of *B. amyloliquefaciens SW19* ethyl acetate extract studied in vitro against pathogenic bacteria (*Aeromonas* spp, *Pseudomonas* spp &*Vibrio* spp), the inhibition zone ranged from (10–45 mm). The obtained result agreed with Habib and Karim

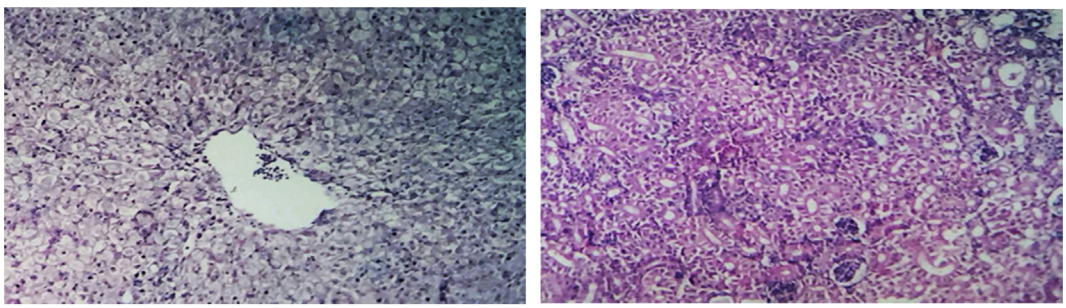

Fig. 4. Photomicrograph (A) of liver of Nile tilapia of the control group (A0) on 60th day showing normal tissue architecture and cellular details within hepatic parenchyma (H&E × 400). Photomicrograph (B): kidney showing normal renal cortex (H&E × 200).

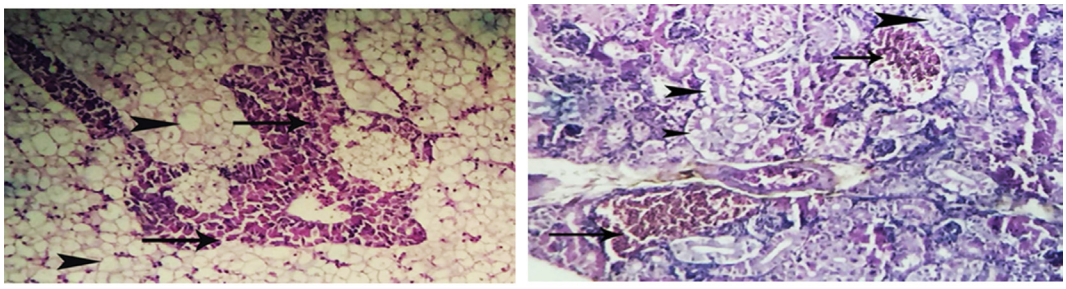

Fig. 5. Photomicrograph (C) of liver of Nile tilapia of group A1 on 60th day showing hyperplasia of hematopoietic tissue invested large blood vessels (arrows) and vacuolation diffuse of hepatocytes (arrowhead) (H&E × 400). Photomicrograph (D) Nile tilapia kidney showing some renal tubules were degenerated (arrowhead) and severe diffuse congestion in the renal blood vessels (arrows) (H&E × 400).

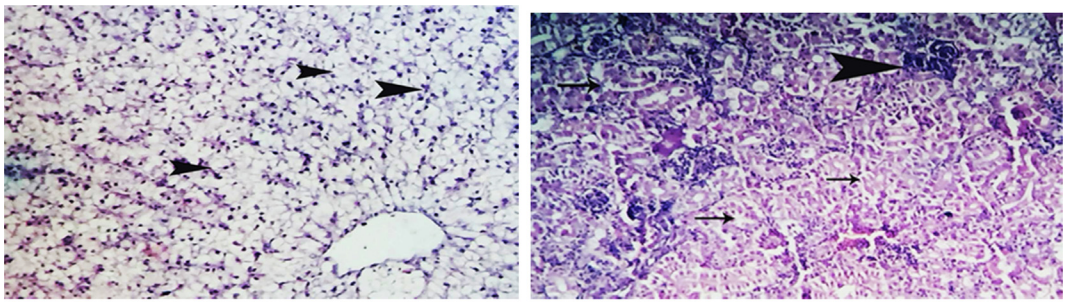

Fig. 6. Photomicrograph (E) of liver of Nile tilapia of the group (A2) on 60th day showing diffuse vacuolation of hepatic cells within hepatic parenchyma (arrowhead) (H&E × 400), Photomicrograph(F) hypercellularity of renal glomeruli (arrowhead)of the kidney and mild degenerative changes at renal tubules (arrows) (H&E × 400).

(2009) demonstrated that ethyl acetate extract of Di-(2-ethylhexyl) phthalate isolated from Calotropis gigantea (Linn.) Flower had antibacterial activity against both Gram positive (*Sarcina lutea, Staphylococcus aureus* and *Bacillus subtilis*) and Gram negative (*Shigella sonnei, Escherchia coli, Shigella dysenteriae and Shigella shiga*) bacteria, inhibition zones ranging from 07 ∼ 20 mm. This antibacterial activity due to the presence of secondary metabolites that can interfere with pathogens by different mode of action, including cell membrane disruption, inhibition the formation of cell wall, prevent the cell division and inhibition the synthesis of RNA/DNA or protein synthesis.

The present investigation pointed that groups of fish were fed dietary supplemented with prebiotics, probiotics, and synbiotics recorded low mortality rates and high survival rates. These results were similar to those obtained by Samuel (2013)**,** who concluded that the probiotic strains and prebiotics used as feed and aquatic additives individually or together effectively reduced mortality in Nile tilapia and channel catfish under the conditions of laboratory.

The current study recorded high percentage of weight gain in groups which received dietary supplementation with synbiotics, followed by groups fed dietary supplementation with probiotics, then groups taking dietary supplementation with prebiotics. The highest (SGR) was obtained in synbiotic groups, while the lowest growth rate and (SGR) were recorded in control. The obtained results agreed with Wibisono et al. (2021)**,** who stated that using synbiotics had an increasing effect on the length and growth of tilapia. It was recorded in group T3 (1 % probiotics and 2 % prebiotics), with 35.36 g weight and 13.17 cm length. Our study cleared that groups that received dietary supplementation with prebiotics, probiotics, and synbiotics showed a significant increase in RBCs, WBCs, and Hb value. The same observation was recorded by Al-Dohail et al. (2011), Reda et al. (2018), Adorian et al. (2019), probiotics could improve hematological parameter value due to hemopoietic stimulation. This agreed with Renuka et al. (2014), widanarni & suprayudi, (2015), and Salam et al. (2021). Our values disagreed to Ruiz et al. (2020), which stated that there were no significant changes at thrombocyte count, total leukocyte, and lymphocytes in Oreochromis niloticus, fed the probiotic *Lactobacillus plantarum* with different concentrations. Higher immunity registered in fish groups fed dietary supplementation with synbiotics, prebiotics, and probiotics is indicated by elevated lysozyme & IgM activities than control diet feeding. In the same direction reported by Abd El-Tawwab and Ahmed (2009) and Abd El-Azeem et al. (2018). The probiotics could produce different cytokines in fish; thus, the immunity system of stomach fish increases the cells of the immunoglobulin acidophil granulocyte (Niu et al. 2020**)**.

Our result demonstrated a significant rise in SOD & CAT in fish groups that received additives of probiotics, prebiotics, and synbiotics and revealed a significant drop in the infected non-treated group. Similar signs were observed by **(**Zhou et al. 2010**),** who mentioned that (*O. niloticus*) enhanced significantly the activity of serum SOD after *B. coagulans* and *B. subtilis* B10 dietary supplemented intake for forty days. The present investigation indicates that a week after the start of the experiment, all treated groups showed a high increase in aspartate aminotransferase (AST) and a considerable reduction in alanine aminotransferase (ALT), albumin, globulin, and total protein. Also, next two month, decreasing significantly ($p \leq 0.05$) in (ALT), while in(AST), total protein, albumin, and globulin were recorded a significant increase in the same groups.Our results were similar to those revealed by Nayak et al. (2007), Kumar et al. (2017), Abd El Azeem et al. (2018), and Zhang et al. (2020), who demonstrated that the activities plasma of ALP, AST, and ALT lowered in groups with xylooligosaccharide supplementation, thus indicates the normality of the XOS liver function protection. Our trial, creatinine & urea were markedly

reduced by dietary supplementation of probiotics, prebiotics, and symbiotics, while increased in groups infected with *V. anguillarium*. This may be attributed to kidney dysfunction and reduction in probiont groups and their roles in improving kidney histology. These results are in the same direction as Sayed et al. (2011), who showed that Nile tilapia (Lin Fingerlings) manifested a significant decrease in urea, creatinine, ALT, and AST in all treated groups with probiotics compared with control.

Histopathological examinations on the different orangs (liver & kidney) of untreated infected Nile tilapia were carried out. The hepatic tissue results indicated necrotic changes, fatty degenerative changes in the pancreatic acini, high degenerative tubular necrosis, and hematopoietic hyperplasia. These results are in the same direction as Fouz et al. (1995), and Azad et al. (2004), who stated that the renal tubules edema, depletion of the tubular interstitium cells, also the main histopathological features in normal and experimentally infected sea bass kidney were hyperemia. In the current study, in the fish groups that received dietary supplementation with probiotics, prebiotics, and synbiotics, the liver of fish clarified a lower degree of damage to cordon side and less of hyperemia compared with non-supplemented ones. Diffuse vacuolation of hepatic cells within hepatic parenchyma suggests the positive effects of probiotics, no harmful inflammatory responses on the immune system (Saad, 2006).

6. Conclusion

The present study exhibited that dietary supplemented with *B. amyloliquefacien* as probiotics plus prebiotics mixed together to obtain synbiotics in Nile tilapia diet for two month could modulate the growth performance and resistance against fish pathogens. Furthermore, it elevated the survival of aquatic animals. However, it could cause some hematological and histological changes.

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.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2022.103539.

References

Abdel Azeem, S., Dowidar, M.F., Khater, A.M., Awad, S.M.M., 2018. Improvement of growth performance, immunity and disease resistance in Nile tilapia, *Oreochromis niloticus*, by using dietary probiotics supplementation. J. Anim. Sci. Vet. Med. 3 (2), 35–46.

Abdel-tawwab, M., Ahmad, M.H., 2009. Live spirulina (*Arthrospira platensis*) as a growth and immunity promoter for Nile tilapia, *Oreochromis niloticus* (L), challenged with pathogenic *Aeromonas hydrophila*. Aquac. Res 40, 1037–1046.

Abumourad, I.M.K., Abbas, W.T., Awaad, E.S., Authman, M.M.N., El-Shafei, K., Sharaf, O.M., Ibrahim, G.A., Sadek, Z.I., El-Sayed, H.S., 2013. Evaluation of *Lactobacillus Plantarum* as probiotic in Aquaculture. Emphasis on growth performance and innate immunity. J. Appl. Sci. Res. 9 (1), 572–582.

Adorian, T.J., Jamali, H., Farsani, H.G., Darvishi, P., Hasanpour, S., Bagheri, T., Roozbehfar, R., 2019. Effects of Probiotic Bacteria Bacillus on Growth Performance, Digestive Enzyme Activity, and Hematological Parameters of Asian Sea Bass, Lates calcarifer (Bloch). Probiotics Antimicrob. Proteins. 11 (1), 248–255.

Akabanda, F., Owusu-Kwarteng, J., Tan, O., Debrah, K., Parkouda, C., Jespersen, L., 2014. The use Lactic acid bacteria starter culture in the production of Nunu, a spontaneously fermented milk production. Ghana Int. J. Food Sci., 271067

Alcaide, E., Blasco, M.D., Esteve, C., 2005. Occurrence of drug-resistant bacteria in two European eel farms. Appl. environ. Microbiol. 71, 3348–3350.

Al-Dohail, M.A., Hashim, R., Aliyu-Paiko, M., 2008. Challenges of probiotic lactobacillus acidophilus against pathogenic bacteria in vitro: expectations and Realizations. Univ. Malaysia Terengganu, 18–20.

Al-Dohail, M.A., Hashim, R., Aliyu-Paiko, M., 2011. Evaluating the use of *lactobacillus acidophilus*. as a biocontrol agent against common pathogenic bacteria and the effects on the haematology parameters and histopathology in African cat fish *Clarias gariepinus* juveniles. Aquac. Res. 42, 196–209.

Aly, S.M., 2009. Probiotics and aquaculture. CAB Rev.: Perspect Agric. Vet. Sci. Nutr Nat. Resour. 4 (074), 1–16.

Aly, S.M., Mohamed, M.F., John, G., 2008. Effect of probiotics on the survival, growth and challenge infection in Tilapia nilotica *(Oreochromis niloticus*). Aquac. Res. 39 (6), 647–656.

Austin, B., Austin, D.A., stin and Austin 2007. Bacterial fish pathogens, Disease of farmed and wild fish. praxis publishing, U. K, p. 581pp.

Azad, I.S., Thirunavukkarasu, A.R., Kailasam, M., Rajan, J.J.S., 2004. Virulence and Histopathology of Vibrio anguillarum like (VAL) Bacterium Isolated from Hatchery Produced Juveniles of Lates calcarifer(Bloch). Asian Fish. Sci. 17, 101–110.

Balcázar, J.L., Rojas-Luna, T., 2007. Inhibitory activity of probiotic *Bacillus subtilis* UTM126 against vibrio species confers protection against Vibriosis in juvenile shrimp (*Litopenaeus vannamei*). Curr. Microbial 55, 409–412.

Bancroft, J.D., Stevens, A., 1996. Theory and practice of histological techniques, 3rd ed. New York.

Barbosa, M.C., Jatoba, F.D.N, Vieira. B.C., Silva, J.L.P., Mourino, E.R., Andreatta, W.Q., Seiffert., Cerqueira, V. R., 2011. Cultivation of juvenile fat Snook (*Centropomus parallelus* poey,1860) Fed probiotic in laboratory conditions ". Braz. Arch. Biol, Technol. 54(4) 795-801.

Cerezuela, R., Meseguer, J., Esteban, M.A., 2011. Current knowledge in symbiotic use for fish Aquaculture: Areview. J. Aquac. Res. Dev. 1, 1–7.

De-Silva, S.S., Anderson, T.A., 1995. Fish nutrition in,. chapman and Hall, London.p, p. 319.

FAO, 2014. Market reports: Tilapia june 2014(internet) (diunduh) 2014 oktober 08. Available at: http://www.globefish.org/tilapia-june-2014-html.

FAO, 2017. FAOSTAT statistics database Food Balance Sheets (2017) Updated Feb 2017). http://www.fao.org/faostat/en/#data/FBS.

Fouz, B., Novoa, B., Toranzo, A.E., Figueras, A., 1995. Histopathological lesions caused by *Vibrio damsela* in cultured turbot, *Scophthalmus maximus* (L.): inoculations with live cells and extracellular products. J. Fish Dis. 18, 357–364.

Fuda, H., Sayano, K., Yamaji, F., Haraj, H., 1991. Serum immunoglobulin M (IgM) during early development of masu salmon (*Oncorhynchus masou*). Comp. Biochem. Physiol. 99A, 637–643.

Gadhi, A.A.A., El-Sherbiny, M.M.O., Al-Sofyani, A.A., Abdulaziz Ba-Akdah, M., Satheesh, S., 2018. Antimicrofouling activities of marine macroalga *Dictyota dichotoma* from the Red Sea. JAMS. 23, 58–67.

Geng, X., Dong, X.H., Tan, B.P., Yang, Q.H., Chi, S.Y., Liu, H.Y., Liu, X.Q., 2012. Effects of dietary probiotic on the growth performance, non –specific immunity and disease resistance of Cobia, *Rachycentron canadum*. Aquac. Nutr. 18 (1), 46–55.

Gibson, G.R., Roberfroid, M.B., 1995. Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. Nutr. J. 125, 1401–1412.

Grisdale-Helland, B., Helland, S.J., Gatlin, W.O.M., 2008. The effects of dietary supplementation with mannanoligosaccharide, fructooligosaccharide or galactooligosaccharide on the growth and feed utilization of Atlantic salmon (Salmosalar). Aquac. 283, 163–167.

Guerreiro, I., Guerreiro, A., Oliva-Teles, P.E., 2018. Prebiotics as functional ingredients: focus on Mediterranean fish aquaculture. Rev. Aquacult. 10, 800–832.

Habib, M.R., Karim, M.R., 2009. Antimicrobial and Cytotoxic Activity of Di-(2-ethylhexyl) Phthalate and Anhydrosophoradiol-3-acetate Isolated from *Calotropis gigantea* (Linn.) Flower. Mycobiology. 37 (1), 31–36.

Hai, N.V., Fotedar, R., 2009. Comparison of the effects of the prebiotics (Bio-Mos and (beta)-1,3-D-glucan) and the customized probiotics (*Pseudomonas synxantha* and *P. aeruginosa*) on the culture of juvenile western king prawns *(penaeus latisulcatus* kishinouye 1896. Aquac. 289, 310–316.

Hong, H.A., Duc, L.H., Cutting, S.M., 2005. The use of bacterial spore formers as probiotics. FEMS Microbial, Rev. 29, 813–835.

Jahari, M.A., Mustafa, S., Roslan, M.A.H., Abd manap, Y., lamasudin, D.U., Jamaludin, F.I., 2018. The effect of symbiotic and probiotics supplementation on growth performance of Red Hybrid Tilapia, *Oreochromis Mossambicus* x *Oreochromis niloticus*. J. BI. M. B. 6 (1):5-9. JO.

Jauncey, K., Ross, B., 1982. A guide to tilapia feeds and feeding. University of Stirling, Stirling, UK.

Kajita, Y., Sakai, M., Atsuta, S., Kobayashi, M., 1990. The immunormodulatory effects of Levamisole on rain bow trout, *Oncorhynchus mykiss*. Fish Pathol 25, 93–98.

Kamoura, N.A., Alazazy, H.M., Abd Elhameid, E.M., Megahed, H.M., 2020. Experimental effect of flavomycin and symbiotic on pseudomonas Aeruginosa infection in Cyprinus carpio (Common carp). Fish. Egypt. J. Comp. Path & Clinic Path. 32 (4), 41–55.

Kim, D., Subramanian, D., Park, S., Jang, Heo. Y.M. 2017. Assessment and Potential Application of the Probiotic Strain, *Bacillus amyloliquefaciens* JFP2, Isolated from Fermented Seafood-Jeotgal in Flounder Paralichthys olivaceus Juveniles. IJA_69:1352-1364.

Krishnan, R., 2014. Probiotic potential of Bacillus species isolated from freshwater fish Anabas testudineus in labeo rohita. Int. J. Multidisc. 1 (1), 46–50.

Kumar, P., Jain, K.K., Sadar, P., Jayant, M., Tok, N.C., 2017. Effect of dietary symbiotic on growth performance, blood composition, digestive enzyme activity and gut Microbiota in Cirrhinus nrigala (Ham)fingerlings. Aqua. Nutr. 24, 921–929.

Liu, H., Qin, S., Wang, Y., Li, W., Zhang, J., 2008. Insecticidal action of Quinomycin A from *Streptomyces sp.* KN.0647, isolated from a forest soil. World.J. Microbiol. Biotechnol. 24, 2243–2248.

Masika, W.S., Moonsamy, G., Mandree, P., Ramchuran, S., Lalloo, R., Kudanga, T., 2020. Biodegradation of petroleum hydrocarbon waste using consortia of *Bacillus* Sp. Bioremediat J., 1–8

McBride, M.J., 2014. The Family *Flavobacteriaceae*. In: Rosenberg, E., DeLong, E.F., Lory, S., Stackebrandt, E., Thompson, F. (Eds.), The Prokaryotes. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-38954-2_130.

Mehrabi, Z., Firouzbakhsh, F., Jafarpour, A., 2012. Effect of dietary supplementation of symbiotic on growth performance, serum biochemical parameters and carcass composition in rainbow trout (*Oncorhynchus mykiss*) fingerlings. J. Anim Physiol. Anim Nutr. (Berl). 96, 474–481.

Muniruzzaman, M., Chowdhuy, M.B.R., 2004. Sensitivity of fish pathogenic bacteria to various medicinal herbs. Bangladesh. J. Vet. Med. 2, 75–82.

Nayak, S.K., Swain, P., Mukherjee, S.C., 2007. Effect of dietary supplementation and vitamin c on the immune response of Indian Major Carp, *Labeo rohita* (Ham). Fish shellfish immunol. 23, 892–896.

Niu, J., Zhao, W., Lu, D., Xie, J., He, X., Fang, H., Liao, S., 2020. Dual-Function Analysis of Astaxanthin on Golden Pompano (Trachinotus ovatus) and Its Role in the Regulation of Gastrointestinal Immunity and Retinal Mitochondrial Dysfunction Under Hypoxia Conditions. Front. Physiol. 11, 568462.

Parry, R.M., Chandan, R.C., Shahani, K.M., 1965. A rapid and sensitive assay of muramidase. Experim Biol. Med. 119 (2), 384–386. https://doi.org/10.3181/00379727-119-30188.

Quade, M.J., Roth, J.A., 1997. A rapid, direct assay to measure degranulation of bovine neutrophil primary granules. Vet. Immunol. Immunopathol. 58 (3–4), 239–248. https://doi.org/10.1016/S0165 -2427(97)00048 -2.

Rajaraman, V., Nonnecke, B., Franklin, S., Hammell, D., Horst, R., 1998. Effect of vitamins A & E on nitric oxide production by blood mononuclear leukocytes from neonatal calves fed milk replacer 1,2,3. J. dairy Sci. 81, 3278–3285.

Reda, R.M., El-Hady, M.A., Selim, K.M., El-Sayed, H.M., 2018. Comparative study of three predominant gut Bacillus strains and a commercial *B. amyloliquefaciens* as probiotics on the performance of *Clarias gariepinus*. Fish Shellfish Immunol. 80, 416–425.

Reed, A.P., Francis-Floyd, R., 1996. Vibrio infection of fish. Florida cooperative extension service institute of food and Agricultural Sciences. FA31.1-3.

Reitman, S., Frankel, S., 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol. 28 (1), 56–63. https://doi.org/10.1093/ajcp/28.1.56.

Rekiel, A., Wiecek, J., Batorska, M., Roszkowskim, T., 2007. Effect of addition of feed antibiotic Flavomycin or prebiotic BIO-MOS on production results of Fatteners, blood biochemical parameters, Morphometric indices of intestine and composition of microflora. Arch. Tierzucht Dummerstorf. 50, 172–180.

Renuka, K.P., Venkateshwarlu, M., Naik, A.R., 2014. Effect of probiotic (*Lactobacillus acidophilus*) on haematological parameters of Catla Catla (Hamilton). Int. J. Curr Microbial. App Sci. 8, 326–335.

Ruiz, M.L., Owatari, M.S., Yamashita, M.M., Ferrarezi, J.V.S., Garcia, P., Cardoso, L., Martins, M.L., Mouriño, J.L.P., 2020. Histological effects on the kidney, spleen, and liver of Nile tilapia *Oreochromis niloticus* fed different concentrations of probiotic *Lactobacillus plantarum*. Trop Anim Health 168 (Prod. 52), 167–176.

Saad, S.M.I., 2006. Probióticos e prebióticos: o estado da arte. Braz. J. Pharm. Sci. 42, 1–16.

Salam, A.M., Islam, M.A., PaulMd, S.I., Rahman, M., Rahman, M.L., Islam, F., Rahman, A., Alam, M., Islam, T., 2021. Gut probiotic bacteria of Barbonymus gonionotus improve growth, hematological parameters and reproductive performances of the host. Sci. Rep., 11 Article number:10692.

Samuel, A. 2013. Effects of pre- and probiotics on pond production, growth and disease susceptibility of channel catfish Ictalurus punctatus and Nile tilapia Oreochromis niloticus". A dissertation submitted to the Graduate Faculty of Auburn University in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

SAS, Institute I NC, 1997. The statistical analysis system for windows 6 – 12 Ed. Cary N. C. USA.

Sayed, S.H., Zakaria, A., Mohamed, G.A., Mohamed, K.K., 2011. Use of probiotics as growth promoter, anti-bacterial and their effects on the physiological parameters and immune Response of *Oreochromis Niloticus* Lin. fingerlings. J. Arab. Aquac. **Soc**. 6 (2), 202–221.

Selim, K.M., El-Sayed, M.H., El-Hady, M.A., Reda, R.R., 2019. In-vitro evaluation of the probiotic candidtes isolated from the gut of *Clarias Gariepinus* with special reference to the in vivo assessment of live and heat-inactivated *Leuconostoc mesenteroides* and *Edwardsiella sp*. Aquac Int. 27, 33–51.

Shaimaa, M.I. 2019. Evaluation probiotic bacteria in bio-controlling Silk warm (BOMBY-X MORI) bacterial infection. A thesis submitted for partial fulfillment of the degree of doctor philosophy of science in microbiology botany and microbiology department faculty of science .Zagazig University.

Siddiqui, A.Q., Howloder, M.S., Adam, A.A., 1988. Effect of dietary protein levels on growth, feed conversion and protein utilization in fry of young Nile tilapia (*Oreochromis niloticus*)". Aquac. 70, 63–73.

Silva, T.A., Petrillo, T.R., Yunis-Aguinaga, J., Fernandes, P., Claudiano, S., Moraes, F.R., Moraes, J.R.E., 2015. Effects of the probiotic Bacillus amyloliquefaciens. on growth performance, hematology and intestinal morphometry in cagereared Nile tilapia. Lat. Am. J. Aquat. Res. 43 (5), 963–971.

Sivarajan, A., Shanmugasundaram, T., Sangeetha, M., Radhakrishnan, M., Balagurunathan, R., 2019. Screening, production, and characterization of biologically active secondary metabolite(s) from marine Streptomyces sp. PA9 for antimicrobial, antioxidant, and mosquito larvicidal activity. Indian J. Mar. Sci. 48 (08), 1319–1326.

Sliva, E.F., Soares, M.A., Calazan, N.F., Vogeley, J.L., Dovalle, B.C., Soares, R., 2013. Effect of probiotic (*Bacillus* spp) addition during larvae and post larvae culture of white shrimp *Litopenaeus Vannemei*. Aquac Res. 44, 13–21.

Tan, H.Y., Chen, S., Hu, S., 2019. Improvements in the growth performance, immunity, disease resistance, and gut microbiota by the probiotic *Rummeliibacillus stabekisii* in Nile tilapia (*Oreochromis niloticus*). Fish Shellfish Immunol. 92, 265–275.

Wibisono, C.B.B., Wahyudi, A.I., Ariefm, M., Lokapirnasari, W.P., 2021. The potency of synbiotics in improving the growth rate, feed conversion ratio, protein retention and lipid retention in Nile tilapia (Oreochromis niunstloticus). AACL Bioflux. 14 (1), 486–494 http://www.bioflux.com.ro/aacl.

Widanarni, D.J.U., Suprayudi, M.A., 2015. Administration of microencapsulated probiotic at different doses to control streptococcosis in Tilapia (*Oreochromis niloticus*). Microbiology. (9) No.1.

Yanbo, W., Zirong, X., 2006. Effect of probiotics for common carp (*Cyprinus carpio*) based on growth performance and digestive enzyme activities. Anim. Feed Sci. Technol. 127 (283-292).

Yousefian, M., Amiri, M.S., 2009. Areview of the use of prebiotic in aquaculture for fish and shrimp. J.AJB. 8 (25), 7313–7318.

Yu, Y., Zheng, Z., Yingeng, W., Meijie, L., Xiaojun, R., Bin, L., Hao, Z., 2019. Effects of Different Preservation Methods on Physicochemical Property of Marine Pathogen Vibrio anguillarum. J. Ocean Univ. China (Oceanic and Coastal Sea Research) 18, 1417–1426.

Zhang, Z., Chen, M., Chen, S.X., Liu, Y., Tian, L., Niu, J., 2020. Effects of dietary xylooligosaccharide on growth performance, enzyme activity and immunity of juvenile grass carp, *Ctenopharyngodon idellus*. Aquac. Rep. 18, 100519.

Zhou, X., Tian, Z., Wang, Y., Li, W., 2010. Effect of treatment with probiotics as water additives on tilapia (*Oreochromis niloticus*) growth performance and immune response. Fish Physiol. Biochem. 36 (3), 501–509.

Further Reading

Hernandez, T., Coulson, R.A., 1967. Amino acid excretion in the alligator. Comp. Biochem. Physiol. 23, 775–784.

Lee, B.J., Kim, S.S., Song, J.W., Oh, D.H., Cha, J.H., Jeong, J.B., Heo, M.S., Kim, K.W., Lee, K.J., 2013. Effects of dietary supplementation of citrus by products fermented with a probiotic microbe on growth performance, innate immunity and disease resistance against *Edwardsiella tarda* in juvenile olive flounder, *paralichthys olivaceus* (TEmminck & Schlegel). J. Fish DIS 36, 617–628.