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Research Article

Amelioration of hemato-serological parameters, antioxidant capacity, and resistance against *Aeromonas hydrophila* in common carp (*Cyprinus carpio*) fed with quorum quenching probiotics

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Abstract

In this study, two probiotics (*Citrobacter freundii* and *Bacillus foraminis*) with indigenous quorum quenching (QQ) were isolated from the intestine of *Cyprinus carpio* and their effects on hemato-serological parameters and innate immune responses, antioxidant capacity and disease resistance in *C. carpio* were determined. The juveniles (n=450, 50.26±10.47 g mean weight) were randomly divided into 6 groups (with 3 replications) and fed with diets containing 1×10^9 cfu g^{-1} of *C. freundii* (QQ1, G1), *B. foraminis* (QQ2, G2), *Lactobacillus plantarum* (without characteristics QQ, WQQ, G3), QQ1+QQ2 (G4), QQ1+QQ2+WQQ (combine, G5), and a control diet (without probiotic) for 60 days. The hematocrit level was increased in the fish fed with QQ1 and WQQ supplemented diets compared to the control group. At 30 days, the combined probiotic treatments (QQ1+QQ2+WQQ) significantly increased hemoglobin and total leukocyte count compared to QQ1 and control, though these differences were not observed at 60 days. QQ2 notably improved complement activity and lysozyme levels after 30 days, while QQ1+QQ2 showed a higher antitrypsin activity at 30 days. The myeloperoxidase levels were elevated in the combined treatments at 60 days. The hepatic catalase activity was higher in QQ probiotic groups at 30 days but it was decreased by 60 days with significant differences noted between WQQ and other groups. The highest superoxide dismutase activity was obtained in QQ2 at 30 and 60 days. The liver glutathione level was decreased in the mixed probiotic groups but it was increased in the combined group at 60 days. A lower content of malondialdehyde was in QQ1 and QQ2 groups at 60 days. The probiotic-treated groups showed significantly lower mortality rates compared to the control against *A. hydrophila* infection, with the highest mortality occurring 30 hours post-infection [G1 (30.6%), G2 (30.6%), G3 (40.63%), G4 (30.6%), G5 (40.63%), and the control group (60.3%)]. Overall, the indigenous probiotics cause an improvement in various health parameters and could reduce mortality rates in infected fish.

Introduction

Aquaculture has been defined in many ways to denote all forms of culturing aquatic animals and plants in fresh and brackish water and the marine environment. The most current accepted definition has been given by the Food and Agriculture Organization as the farming of aquatic organisms including fish, crustaceans, molluscs and aquatic plants (Palma and Viegas, 2022). The global production of fish via aquaculture and capture fisheries is estimated to have reached about 179 million tonnes in 2018. Of the 82.1 million tonnes of aquatic animals produced by aquaculture sectors, about 4.19 million tonnes belong to common carp (*Cyprinus carpio*) and about 0.21 million tonnes belong to production of Iran (FAO, 2020). Common carp is one of the economically important freshwater fish that is usually reared in earthen ponds. Carp species can be produced even in lower-quality waters, which is an especially valuable characteristic in the Asian and Middle east regions. From the aspect of sustainability achieved in carp pond, it is essential to perform fish meal-independent and cereal-based fish meat production which provides increasing production of this fish species in the long run. One of the problems of carp culture in the world is the high mortality due to various diseases. FAO has shown that the use of probiotics is one of the best ways to increase the resistance and safety of fish and reduce losses due to stressors (Mohammadian *et al*., 2019). In this context, it has been reported that the highest cost paid for carp production in Iran is related to feed. Moreover, probiotics have beneficial impacts on the host due to the inhibition of the growth of pathogens (Gatesoupe, 1999), alteration of antioxidant capacity and stimulation of immune responses (Shefat, 2018). Likewise, use of probiotics is an eco-friendly and sustainable approach that reduces the use of harmful chemical compounds particularly antibiotics in aquaculture (Gatesoupe,1999). The *Aeromonas hydrophila* is one of the most important gram-negative pathogenic bacteria in fish that causes hemorrhagic septicemia, ascites and mortality at different ages and in different species of fish (Mzula *et al*. 2019). This bacterium is considered one of the most important health problems in fish farming in different countries. On the other hand, the increasing growth of aquaculture has caused the spread of more and more diseases, including infection with this bacterium (Alishahi *et al*, 2022). Antibiotic resistance has been increasingly reported for a wide variety of bacteria of clinical significance. This widespread problem constitutes one of the greatest challenges of the twenty-first century. Faced with this issue, clinicians and researchers have been persuaded to design novel strategies in order to try to control pathogenic bacteria. Therefore, the discovery and elucidation of the mechanisms underlying bacterial pathogenesis and intercellular communication have opened new perspectives for the development of alternative approaches. Antipathogenic and/or antivirulence therapies based on the interruption of quorum sensing pathways are one of several such promising strategies aimed at disarming rather than at eradicating bacterial pathogens during the course of colonization and infection

(Ghanei-Motlagh *et al*., 2019). Quorum sensing (QS) is a process by which bacteria monitor their population in a cell-density dependent manner through the synthesis, exchange, and the detection of small intracellular signals (autoinducers) (Xavier and Bassler, 2005) Among the various types of signals that are produced in bacteria (Autoinducer-1, Autoinducerprobably the best studied. Bacteria produce the QS molecules, and when the intensity of these signals reaches a threshold, they diffuse back into the bacterial cell and regulate the expression of QS-related genes such as those involved in biofilm formation and production of virulence determinants (Galloway *et al.,* 2011). Indeed, QS allows bacteria to operate as multicellular organisms. Since AHL-based QS is common among freshwater *Aeromonas*, improving our understanding of the procedures related to QS generation by *Aeromonas* and techniques to disrupt these mechanisms will provide new eco-friendly methods for the prevention and treatment of *Aeromonas* infections (Case *et al*., 2008). Quorum quenching (QQ) is a phenomenon opposite to QS and refers to the inactivation of the chemical signals via a mechanism mediated by a number of enzymes, including AHL lactonase, AHL acylase, and AHL oxidoreductase (Nain *et al*., 2020). As an anti-infective strategy, QQ may also compel less selective pressure on pathogens, and in turn, delay the development of resistance mechanisms (Shastry *et al*., 2018). Application of AHL degrading bacteria able to utilize AHL molecules of marine and freshwater pathogens as a food source is a developing idea due to their previously successful outcomes, however, the majority of the strains that have been tested in the past are allochthonous QQ bacteria (Ghanei-Motlagh *et al*., 2019). Moreover, the isolation of autochthonous bacteria with QQ potential has frequently been reported from freshwater fish. On the other hand, despite the high mortalities caused by freshwater *Aeromonas*, QQ strategy has not been adopted against commonly occurring *Aeromonas* pathogens in fish, particularly, *C. carpio*, an adaptive freshwater fish with high economic importance that has gained much attention from researchers and farmers in the last decade. In contrast with some probiotics that are able to out-compete pathogens through the production of antimicrobials, QQ probiotics are neither bactericidal nor bacteriostatic against the targeted pathogens. Instead, they disrupt QS signals and a affect the pathogenicity of their target. On the contrary, one of the main criteria for screening potential probiotic candidates is the inhibitory activity against different pathogens (van Kessel *at el*., 2016). However, there is no comprehensive investigation of the real probiotic characterization of QQ bacteria prior to their administration to fish farm culture and their application on a commercial scale. In the present study, QQ bacteria with a potential to degrade the dominant range of AHL molecules produced by several significant and prevalent pathogenic *Aeromonas* spp. in fish, were isolated from the intestine of common carp and characterized and their efficacy as autochthonous probiotics was tested for the first time. The present investigation was undertaken to evaluate

the changes in various immune parameters of common carp after feeding them with QQ bacteria isolated from theirintestine and also their resistance to a virulent strain of *A. hydrophila*.

Materials and methods

Bacteria

Bacterial isolates were recovered using a previously described method (Irianto and Austin, 2002). Briefly, the entire digestive tracts of *C. Carpio* captured from natural water resources of Khuzestan province (Abzi Company from Dezful) in Iran were removed and their contents were discarded. The quorum quenching potential of *C. freundii* QQ1 and *B. foraminis* QQ2 was confirmed in our previous study using the agar well diffusion and thin layer chromatography methods. In this study, their QQ activity was also tested against *Yersinia ruckerie* by the degradation assay on Luria-Bertani agar as suggested by Chu *et al*. (2011). The tested *Y. ruckerie* was able to induce *Chromobacterium violaceum* CV026. This biosensor respondes to exogenous AHLs with *N*-acyl side chains from C_4 to C_8 in length with production of purple pigment violacein. *Pseudomonas fluorescence* P3/pME6863 and *Pseudomonas fluorescence* P3/pME6000 were respectively used as positive and negative controls in AHL degradation assay. The strains CV026, P3/pME6863 and P3/pME6000 were kindly provided by Dr. Torabi Delshad. The *L. plantarum* strains used in this study as none QQ character were primarily identified based on colony and cell morphology, Gram staining, biochemical characteristics, and 16S rRNA gene sequencing (GenBank accession number EU520326 and EU520327) (Mohammadian *et al*., 2016). These strains were grown for 30 h at 37˚C in MRS broth (BD Difco, Sparks, MD, USA).

Diet preparation

The control diet was formulated using the ingredients as subsequently described. The proximate analysis of the basal diet according to the AOAC method was: 37.1% for crude protein, 8.8% for crude lipid, 9.6% ash and 390 Kcal per 100 g for gross energy. Probiotic bacterial suspensions were prepared by centrifuging (15min., 4000 rpm) the 72h TSB cultured bacteria and resuspending them in PBS at the concentration of Macfarland grade 3 (1.2×10^9 cfu mL^{-1}), The probiotic-enriched diets were prepared by gently spraying of the prepared bacterial suspension on the control and mixing that part by part in a drum mixer to obtain a final probiotic concentration of 1×10^9 $cfug^{-1}$. They were packed in sterile propylene containers and stored at 4˚C for viability studies for a week. This dose was chosen based on a previously recommended dose (Takafoyan *et al*., 2024). The final concentrations of probiotic bacteria in the diet were confirmed by suspending one gram of food in sterile PBS and culturing the serial diluted food suspension in TSA media. Counted bacteria in the food were almost the same as added probiotic bacteria in all batches of probiotic-enriched diets.

Experimental design

Juveniles of *C. carpio* (50±10 g) were transferred from a private cyprinid farm in Khuzestan Province, Iran, to the Lab of

Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz. The fish were acclimated for 2 weeks in indoor 300 L fiberglass tanks and were fed with a standard diet (37.1% crude protein, 8.8% crude lipid, 9.6% ash, and 390 Kcal $100g^{-1}$ gross energy). Then, after verifying the health status of the fish, they were distributed randomly into 12 tanks at an initial density of 25 fish per tank and divided into 6 treatment groups, including control (n=25), QQ1 (G1, n=25), QQ2 (G2, n=25), *L. plantarum* (G3, n=25 as a without characteristics QQ), QQ1+QQ2 (G4, n=25), QQ1+QQ2+W QQ (G5, n=25). Final concentration of each probiotic was about 1×10^9cfu g^{-1} of the diet (Table 1) (Nikoskelainen *et al*., 2001). The aquaria were supplied with water from external Biofilteres (Athmann, China), at a temperature of 25.9±1.2°C. The fish were fed with probiotic-contained diets for 60 days (twice a day). During the experimental period, the temperature ranged from 24.5 to 28.5°C, salinity was from 0.6±0.11 % and the dissolved oxygen was 5.9±1.3mgL^{-1}.

Table 1: The experimental design and treatment setting up, applied in this study.

Treatment	G1	G2	G3	G4	G5	Control
Probiotics category	QQ1	QQ2	*L. plantarum* (W QQ)	QQ1 + QQ2	QQ1 + QQ2+W QQ	Normal saline
Additive quantity (g/kg)	1×10^9	1×10^9	1×10^9	1×10^9	1×10^9	0.0

Sample collection

Samples were collected at 30 and 60 days from the beginning of experiment. At these times, the fish were anesthetized with 2-phenoxyethanol (0.3 mL/L), Blood samples were withdrawn from the caudal vein of four fish per aquarium using a 2.5-mL syringe. One part of the collected blood was dropped in a heparinized microtube and the residue was subjected to centrifugation (30009g, 10 min, 4°C) to separate serum. The sera were then frozen at -80°C until use (Ghanei-Motlagh *et al*., 2021).

Hemato-immunological parameters

Heparinized blood was used to measure the hematological indices. To estimate Hematocrit (Hct), blood was transferred from the eppendorf tubes to plain microcapillary tubes, up to about 75% of the tubes' capacity and the lower opening of the tubes was plugged by capillary clay. They were centrifugated in a capillary microcentrifuge at 12,000 ×*g* for 5 min (Hematocrit centrifuge, Hettich, Germany) and the percentage of Hct was reported using a hematocrit reader. The hemoglobin (Hb) concentration was measured using the cyanomethemoglobin method (Drabkin, 1950).

Total erythrocyte count (TEC) and total leucocyte count (TLC) were simultaneously carried out using an improved Neubauer hemocytometer (Kerr, 2008). To address that, 5 µL of each blood sample were removed using a tip pre-attached to a micropipette. The outside of the pipette tip was wiped from any excess blood with a lint-free cloth, and then blood was diluted in 995 µL (dilution factor = 200) of Natt-Herricks staining solution at room temperature for 5 min (Natt and

Herrick, 1952). Natt-Herricks solution (3.88 g NaCl, 2.5 g Na_2SO_4, 2.91 g Na_2HPO_4-$12H_2O$, 0.25 g KH_2PO_4, 7.5 ml formalin 37%, 0.1 g crystal violet per 1000 ml distilled water, final pH: 7.3) was previously prepared and filtered using a Whatman filter paper (No. 2) after overnight incubation. Thereafter, 10 µL of the diluted sample were loaded on the hemacytometer counting chamber and the cells were allowed to settle in a pre-humid petri dish for 3-5 minutes. TEC was performed in 5 squares (the middle square and 4 corner squares) within the large center square of the chamber using a light microscope (40× objective) under lowered condenser mood, while TLC was carried out in the 4 large corner squares of the chamber at 100× magnification (10× objective) under the same mood. Subsequently, TEC and TLC were calculated by means of the following formula:

TEC ($\times 10^6/\mu L$)= (total number of red blood cells counted × dilution factor) / volume of squares counted (mm^3)
Where, the volume of squares counted was calculated by multiplying the total dimensions of the squares counted (0.2 mm^2) to the distance between the coverslip and chamber (0.1 mm)

TLC ($\times 10^3/\mu L$)= (total number of white blood cells counted × dilution factor) / volume of squares counted (mm^3)
Where, the volume of squares counted was measured by multiplying the total dimensions of the squares counted (4 mm^2) to the distance between the coverslip and chamber (0.1 mm)

Innate immune parameters

Serum lysozyme activity was measured in turbidity as described by Ellis (1990). Serum anti-protease activity was performed by incubating 10 µL of serum with 20 µg of trypsin dissolved in 100 µL of Tris-HCl (50 mM, pH 8.2). In serum blank, 100 µL of Tris-HCl was added to 10 µL of serum, instead of trypsin in Tris-HCl, and in the positive control, no serum was added to trypsin. All tubes were made up to 200 µL with Tris-HCl and incubated for 1 hour at room temperature. After the incubation, 2 ml of 0.1 mM substrate BAPNA (Na-benzoyl-DL-arginine-*p*-nitroanilide HCl, Sigma chemicals), dissolved in Tris-HCl (containing 20 mM calcium chloride), was added to all tubes and incubated for further 15 minutes. At the end of incubation, the reaction was stopped by adding 500 µL of 30% acetic acid. The optical density was measured at 410 nm by using a UV-Visible spectrophotometer (Shimadzu UV-1601) (Sareyyupoglu *et al*. 2010). Total protein and albumin concentrations were determined (Zist Shimi kit, Iran) according to Nayak *et al*. (2010). The albumin content was estimated spectrophotometrically using a standard kit (Glaxo, India). The globulin content was estimated by subtracting the albumin content from the total protein content. In order to assess myeloperoxidase (MPO) activity, 15 µL of serum was diluted in 135 µL of Hanks' balanced salt solution (HBSS, modified without Ca2+ an Mg2+) in a 96-well plate. Subsequently, 25µL of 3,3′,5,5-tetramethylbenzidine (TMB, 20 mM,

Sigma-Aldrich) and 25 µL of diluted hydrogen peroxide (H2O2, 5 mM, Sigma-Aldrich) were added to each well. The reaction was stopped precisely after 2 min by adding 25 µL of diluted sulfuric acid (H2SO4, 4 M, Sigma-Aldrich) and the optical density was measured at 450 nm in a microplate reader (Mohapatra *et al*., 2014).

Oxidative stress indicators

The serum SOD (*Superoxide dismutase*) activity was determined using a commercial kit (RANSOD kit, Randox Com, UK) on a microplate-reader (Synergy HT, BioTek, USA) according to the manufacturer's protocol, which measured the conversion of superoxide anion to hydrogen peroxide.

The activity of CAT (Catalase) was assayed according to the method described by Koroliuk *et al*. (1988). Serum CAT was determined based on the decomposition rate of hydrogen peroxide. Briefly, 5 µL of the serum sample, 100 µL of H_2O_2 (10 mM), 50 µL of Tris HCl buffer (50 mM, pH=7.8), and were poured into microplate wells. After 10 min at room temperature, 100 µL of 4% ammonium molybdate was added to wells. Then, the optical absorption rate of the samples against the control by a microplate analyzer was read at 410 nm and expressed as IU/mg protein.

The serum GSH (*Glutathione*) activity was assayed using the method of Ellman (1959). Briefly, the reaction solution consisted of 15 µL of the serum sample, 260 µL of sodium phosphate buffer (Na2HPO4, 0.1 M) containing EDTA (1 mM, pH=8) and DTNB reagent (0.01 M). 5 µL of Elman's reagent was added to this solution. Then, this solution was incubated at room temperature for 15 min and then read at 412 nm against the blank sample using the microplate reader. The GSH activity was stated as µmol/L serum.

The serum MDA (*Malondialdehyde)* level was measured according to the method of Mihara and Uchiyama (1978). Briefly, 25 µL of each sample was mixed with the working solution (250 ml of 20% trichloroacetic acid (TCA) and 100 ml of 0.6% TBA) and kept in a hot water bath for 30 min. The solution after cooling was centrifuged at 5,000g for 5 min. After that, the samples optical absorption was detected spectrophotometrically at 535 nm. Finally, the results were expressed as nmol/mg protein.

Challenge test

The *A. hydrophila*, AH04, was grown in nutrient broth and incubated at 37^C for 24 h. Lethal dose 50 % (LD50) of *A. hydrophila* (i.e., able to kill 50 % of the *C. carpio* population) was determined by intraperitoneal injection of 60 fish with different doses of *A.hydrophila* (10^6,10^7,$10^8$and 10^9CFU/fish) at 25°C. LD50 was calculated using the method described by Reed and Muench (1938) with consideration of Koch's postulate. Sixteen days after experimental feeding, the fish from each group were divided into two subgroups (45 fish in each group), one as a challenge group to be infected with *A. hydrophila* and the other as an uninfected control. The challenge group was injected intraperitoneally with 0.2 mL PBS containing the LD50 dose of live *A. hydrophila* determined above, while control fish received only 0.2 ml PBS. The

cumulative mortality was determined after 10 days post-infection (Mohammadian *et al*., 2016).

Statistical analysis

All statistical tests were performed using SPSS software (SPSS, Release 16.0, SPSS, Chicago, IL, USA). Two-way analysis of variance (ANOVA) and a general linear model were used to evaluate the effect of time and treatments on each variable. One-way analysis of ANOVA was done to determine the differences between different variables. Differences were considered statistically significant when $p<0.05$ and the results are expressed as mean±SD (Mohammadian *et al*., 2016).

Result

Hematological indices and respiratory burst activity

As presented in Table 2, Hct was significantly enhanced in *C. carpio* supplemented with QQ1 and WQQ for 60 days compared to the control groups ($p<0.05$).

Table 2: Hematological indices of *C. carpio* fed either regular feed or feed supplemented with probiotics for 60 days.

Parameters	Groups	Day 30	Day 60
Hct (%)	QQ1	37.5 ± 6.4^{Aa}	40.1 ± 2.7^{Aa}
	QQ2	39.9 ± 4.11^{Aa}	32.36 ± 3.52^{ABa}
	WQQ	35.6 ± 3.52^{Aa}	39.3 ± 2.5^{Aa}
	QQ1+QQ2	30.17 ± 2.2^{Aa}	35.3 ± 1.5^{ABa}
	Combine	$34.\ 4\pm6.32^{Aa}$	29.73 ± 4.15^{Ba}
	Control	34.8 ± 3.262^{Aa}	30.36 ± 1.5^{Ba}
Hb (g/dl)	QQ1	29.2 ± 6.4^{ABa}	15.5 ± 8.7^{Ab}
	QQ2	28.96 ± 4.11^{ABa}	14.27 ± 2.56^{Ab}
	WQQ	27.1 ± 4.52^{ABa}	11.43 ± 3.55^{Aa}
	QQ1+QQ2	19.7 ± 3.2^{Ba}	13.5 ± 4.9^{Ab}
	Combine	33.54 ± 11.32^{Aa}	13.16 ± 5.55^{Ab}
	Control	26.8 ± 2.262^{ABa}	11.03 ± 3.9^{Ab}
TEC ($\times10^6/\mu L$)	QQ1	$2.6\pm0.26^{A,b}$	$4.07\pm0.12^{A,a}$
	QQ2	$2.5\pm0.11^{A,b}$	$3.28\pm0.6^{A,a}$
	WQQ	$2.86\pm0.32^{A,b}$	$3.98\pm0.01^{A,a}$
	QQ1+QQ2	$2.62\pm0.2^{A,b}$	$3.54\pm0.25^{A,a}$
	Combine	$2.72\pm0.22^{A,a}$	$3.24\pm0.1^{A,a}$
	Control	$2.62\pm0.12^{A,a}$	3.098 ± 0.05^{Aa}
TLC ($\times10^3/\mu L$)	QQ1	$1.25\pm0.04^{AB,a}$	$1.06\pm0.1^{A,a}$
	QQ2	1.05 ± 0.09^{ABa}	$1.087\pm0.05^{A,a}$
	WQQ	$1.41\pm0.02^{A,a}$	$1.04\pm0.01^{A,b}$
	QQ1+QQ2	$0.51\pm0.06^{B,b}$	1.02 ± 0.1^{Aa}
	Combine	$1.08\pm0.02^{AB,a}$	$1.05\pm0.01^{A,a}$
	Control	0.83 ± 0.06^{Ba}	$1.01\pm0.01^{A,a}$

***** For each parameter, values (Mean ± SD) bearing different uppercase letters or different lowercase letters represent significant differences within each column or each row, respectively ($p<0.05$). Hct: hematocrit, Hb: hemoglobin, TEC: total erythrocyte count, and TLC: total leucocyte count.

A significant increase in Hb content was observed in the combined (QQ1+QQ2+WQQ) probiotic treatments when compared to the QQ1 and control groups at day 30, whereas Hb was not significantly differences between

treatments at day 60 (p>0.05). A significant increase in TLC content was observed in WQQ treatment when compared to the QQ1+QQ2 and control groups at day 30, whereas TLC was not significantly different between treatments at day 60 (p>0.05). After 30 days of feeding the probiotic, the complement activity in the groups fed with the QQ2 was significantly increased in relation to the control and the other groups (P<0.05). At 60 day of probiotic feeding, only the QQ1+QQ2 group had a significant difference in complement activity with respect to QQ1 (p<0.05). At 30 day of probiotic feeding, QQ1+QQ2 group had higher serum lysozyme activity than that of other groups (P<0.05). At the end of the experimental period, only the QQ1 and QQ2 groups had a significant difference in lysozyme activity with the control group (P<0.05). The total plasma globulin levels were not different observed between treatments (Fig. 1; p>0.05).

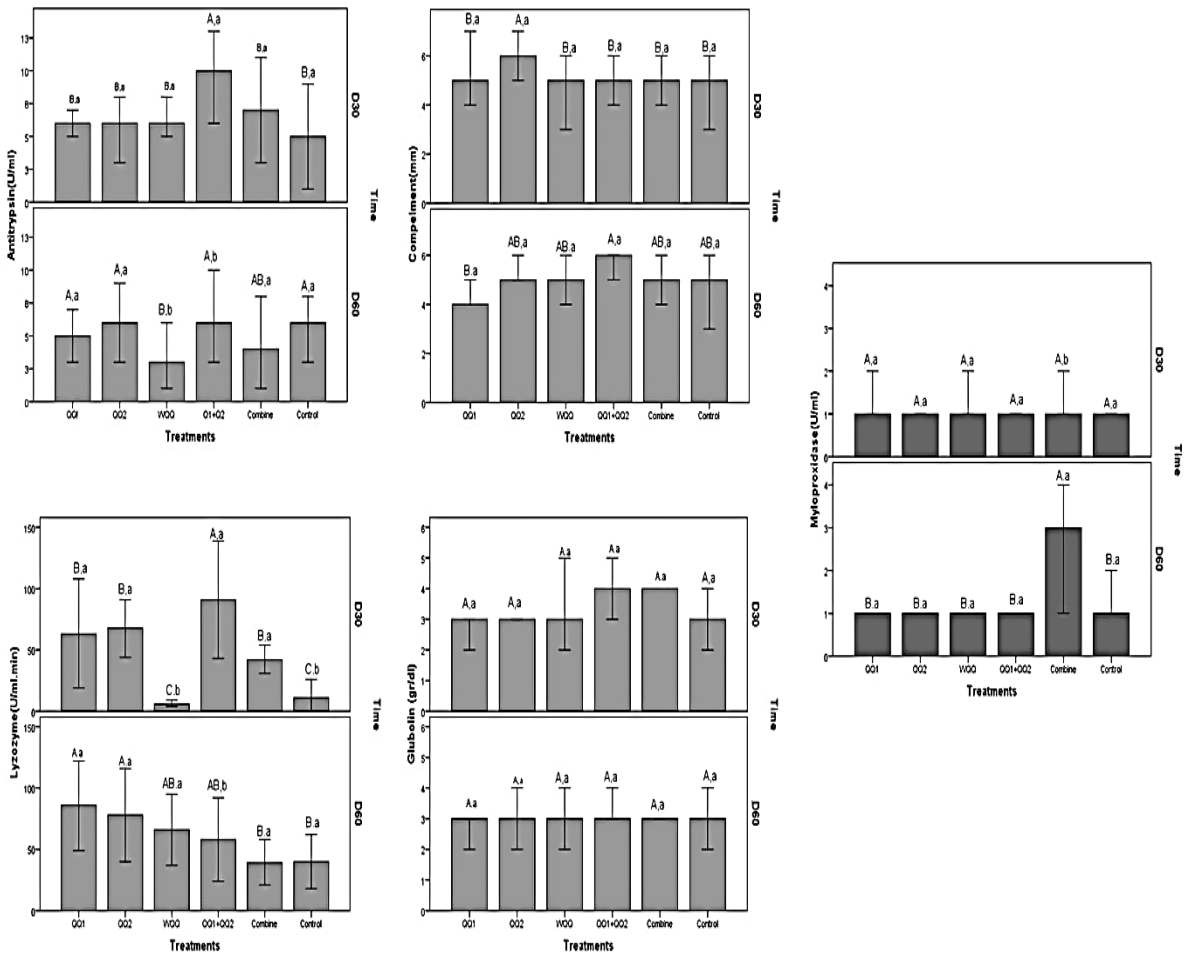

Figure 1: Innate immune parameters activity in *Cyprinus carpio* treated with QQ1 (*C. freundii* ~1×10^9 cfu g^{-1}), QQ2 (*B. foraminis* ~1×10^9 cfu g^{-1}), WQQ (*Lactobacillus planturum* ~1×10^9 cfu g^{-1}), QQ1+QQ2 (*C. freundii* and *B. foraminis* ~1×10^9 cfu g^{-1}), QQ2 (*B. foraminis* ~1×10^9 cfu g^{-1}), QQ1+QQ2 +WQQ (*C. freundii* and *B. foraminis and Lactobacillus planturum* ~1×10^9 cfu g^{-1}) and without probiotic (control). Values is shown as means±standard error (n=9). Legends as mentioned in Figure 1. *For each parameter, values (Mean ± SD) bearing different lowercase letters or different uppercase letters represent significant differences within each column or each row, respectively (p<0.05).

At 30 day of probiotic feeding, QQ1+QQ2 group had higher serum Antitrypsin activity than that of other groups (p<0.05). At the end of the experimental period, only the WQQ group had a significant difference in Antitrypsin activity with the other groups (except for the combine group) (p<0.05). A significant increase in myeloperoxidase content was observed in combine (QQ1+QQ2+WQQ) probiotic treatments when compared to the other groups at day 60, whereas myeloperoxidase was no significant differences between treatments at day 30 (p>0.05).

Liver oxidative stress indicators

The data in Figure 2 describe the liver antioxidant capacity of *C. carpio* fed with QQ probiotics. After 30 days of feeding, liver CAT activities were enhanced (p<0.05) in the QQ probiotic groups compared to the mixed probiotic groups (QQ1+QQ2 and combine), while no significant (p>0.05) difference was observed in their levels between the probiotic treatments and control group. After 60 days of feeding, liver CAT activities were decreased (p<0.05) in all groups compared to 30 days of feeding, while a significant (p<0.05) difference was observed in level between the WQQ treatments and other groups. An enhancement in liver SOD activity was observed in the QQ2 and WQQ groups compared to the control group at days 30 and 60, however, the highest activity (p<0.05) was noticed in fish received *B. forminis* (QQ2). However, the WQQ groups displayed lower SOD activities in days 60 (p<0.05) relative to the days 30. After days 30 the level of GSH in the liver showed a reduction in all mixed groups relative to separated groups. Nevertheless, liver GSH at day 60 was significantly higher in combine group than the days 30. Moreover, MDA content in the liver was significantly (p<0.05) lower in the QQ1 and QQ2 groups compared to the QQ1+QQ2 group after 60 days (Fig. 2).

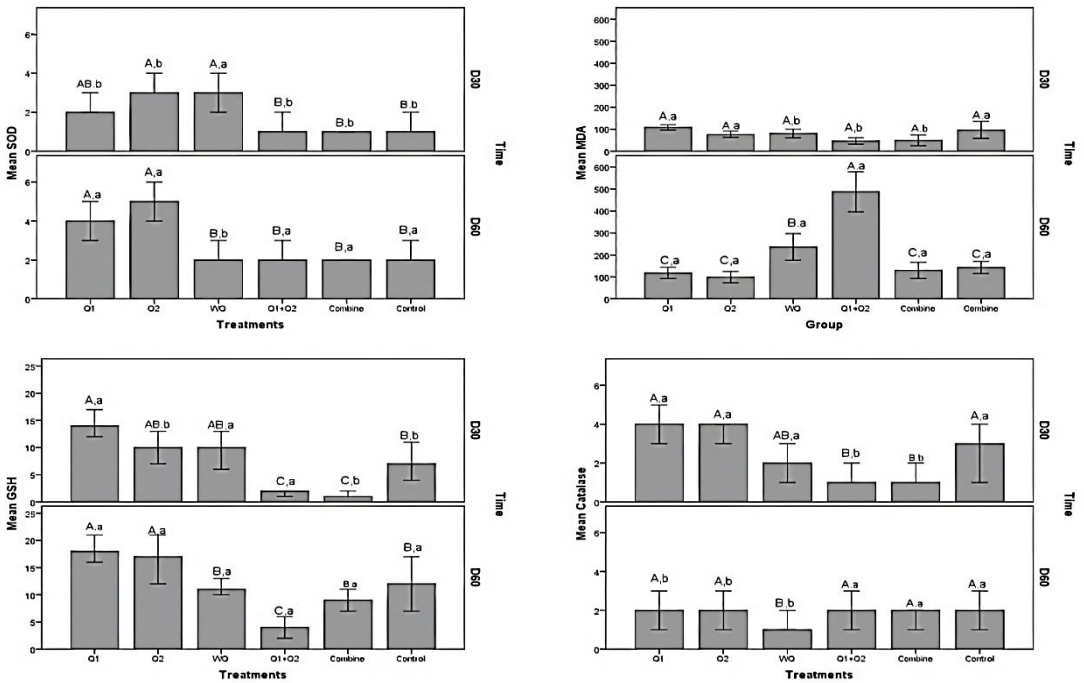

Figure 2: liver antioxidant capacity in *Cyprinus caroio* treated with QQ1 (*C. freundii* ~1×10^9 cfu g^{-1}), QQ2 (*B. foraminis* ~1×10^9 cfu g^{-1}), WQQ (*Lactobacillus planturum* ~1×10^9 cfu g^{-1}), QQ1+QQ2 (*C. freundii* and *B. foraminis* ~1×10^9 cfu g^{-1}), QQ2 (*B. foraminis* ~1×10^9 cfu g^{-1}), QQ1+QQ2 +WQQ (*C. freundii* and *B. foraminis and Lactobacillus planturum* ~1×10^9 cfu g^{-1}) and without probiotic (control). Values are shown as means±standard error (n=9). Legends as mentioned in Figure 2. *For each parameter, values (Mean ± SD) bearing different lowercase letters or different uppercase letters represent significant differences within each column or each row, respectively (p<0.05).

Challenge test

Analysis of the rate of mortality showed that the LD50 on day 10 was 3.7×10^8 CFU/fish. After the challenge test, there were significant differences in the cumulative mortality rate among probiotic-treated and control groups. The first mortalities due to *A. hydrophilla* began at 24 h post-infection (hpi) in all infected groups. The cause of death was confirmed as *A. hydrophila* by bacterial isolation from the spleen and head kidney. Mortalities continuously occurred until 72 hpi. The highest mortality rate was recorded in the control group. Cumulative mortalities were significantly lower in the probiotic-treated groups than the control group (60.3% mortally) (Table 3).

Table 3: Mortality rate percentage in different treatments after challenge with *Streptococcus iniae*.

Parameters	Treatments					
	QQ1	QQ2	*L.Plantaum* (WQQ)	QQ1+QQ2	Combine	Control
Mortality rate (%)	30.6[a]	30.6[a]	40.63[a]	30.6[a]	40.63[a]	60.3[b]

The data represent the Mean±SD of three tanks per treatment. Values with various lowercase letters in each row indicate significant differences (p<0.05).

Discussion

Probiotics can modulate the host immune responses through interaction with immune components within the gut or mucus, triggering immune-related signaling pathways and their effects on the production of cytokines (Thy *et al*., 2017). According to our results, at 30 days, the QQ1+QQ2 group had significantly higher serum lysozyme activity compared to other groups, and by the end of the experiment, significant lysozyme differences were seen between the QQ1, QQ2, and control groups. Lysozyme activity was significantly higher in the QQ1+QQ2 group at 30 days, and the highest activity was observed in QQ1 and QQ2 at day 60. Lysozyme is a bacteriolytic enzyme that directly hydrolyze the peptidoglycan layer of Gram-positive bacteria. It has also a lytic activity against Gram-negative bacteria after damage by complement system which exposes the peptidoglycan cell wall (Nakanishi *et al*., 2018). Furthermore, as an opsonin, lysozyme activate phagocytes and the complement system. Our results are in line with those of Jiang *et al.* (2019) who reported that administration of dietary *B. cereus* QSI-1 at 5×10^8 CFU/g feed for 15 days could enhance lysozyme and complement activities in skin mucus of crucian carp. In this study, a reduction in lysozyme activity was observed in combine, as compared the other groups. Similar results were observed, dietary probiotic increased serum lysozyme activity in striped snakehead (*Channa striata*) (Talpur *et al*., 2014); Asian sea bass (*L.calcarifer*) (Siddik *et al*., 2022; Mozanzadeh *et al*., 2023), *E. bruneus* (Harikrishnan *et al.*, 2010), *E. coioides* (Son *et al*., 2009), Shabot *(Tor grypus*) (Mohammadian *et al*., 2016) and *Labeo rohita* (Giri *et al*., 2013).

Serum complement activity is an important humoral component of the non-specific immune system with bactericidal, chemotactic and opsonic activities (Budi˜

no *et al*., 2006). Lipopolysaccharides from Gram-negative bacteria and foreign red blood cells are among the known activators of this pathway (Nakanishi *et al*., 2018; Ghanei-motlagh *et al*., 2021). In this study, serum complement activity was significantly increased in fish fed with dietary QQ2 at 30 days. It has been previously reported that dietary *B. aerius* B81e significantly increased serum complement, lysozyme, bactericidal activities and RB in Pla-Mong (*Pangasius bocourti*), in a manner consistent with our present results (Meidong *et al*., 2018). After day 60, complement activity was higher in the QQ1+QQ2 groups compared to the QQ1 group. In accordance with our results, a higher compelment activity was reported in Asain sea bass (Mozanzadeh *et al*., 2023), *Salmo trutta caspius* (Hoseinpouri Ghasemabad Sofla *et al*., 2024) fed with mix probiotics, and olive founder (*Paralichthys olivaceus*) fed with diets containing probiotic and herbal extract (Harikrishnan *et al*., 2011).

Serum protease inhibitors are involved in acute phase reactions, homeostasis of the body fluids and defense against protease-producing bacteria (Magnad´ ottir, 2006). Our findings revealed that serum antiprotease activity was higher in fish fed with the mixed QQ probiotics (QQ1+QQ2) at days 30. In agreement with our results, Nile tilapia fed with diets containing *B. subtilis* and *B. licheniformis* had significantly enhanced levels of serum antiprotease in comparison with the un-treated fsh (Abarike *et al*., 2018). Moreover, at the end of this study, the WQQ group showed a significant difference in antitrypsin activity compared to all groups except the combined treatment group. In agreement with our result, feeding with dietary *L. sakei* BK19 promoted several immune parameters including serum antiprotease activity in fish (Harikrishnan *et al*., 2011) also use of *Bacillus subtilis* AB1 in the diet of rainbow trout (*O. mykiss*) for two weeks could significantly increase antiprotease activity (Newaj-Fyzul *et al*., 2007). Likewise, olive flounder (*Paralichthys olivaceus*) fed with *E. fascium* for 5 days had an increased levels of serum antiprotease activity (Kim and Kim, 2013).

Myeloperoxidase (MPO) is a major enzyme present in azurophilic (primary) granules of granulocytic cells (mostly neutrophils) and modulates the immune response and inflammatory processes. MPO is involved in both oxidative (chlorination and peroxidase activities) and antioxidant (superoxidase, SOD and CAT activities) activities. Under normal conditions, MPO and its derivatives provide an effective microbicidal system against pathogens. However, excessive production of MPO-derived oxidants has been associated with several pathological circumstances in higher-developed organisms (Winterbourn and Kettle, 2013). According to the results, a higher serum MPO activity was recorded in the combined treatment group (QQ1+QQ2+WQQ) compared to the control group at day 60. Consistent with our results, Zhang *et al*. (2017) showed that supplementation with *Lactobacillus delbrueckii* led to a significant elevation in the lysozyme and MPO activities compared to the control group. Also, improvement of the above-mentioned immune responses has been

documented following oral administration of probiotics in Nile tilapia (Makled *et al*., 2019), pirarucu (*Arapaima gigas)* (Pereira *et al.,* 2019), catla (*Catla catla*) (Bhatnagar and Saluja, 2019), red seabream (*Pagrus major*) (Dawood *et al*., 2019), striped catfsh (*Pangasianodon hypophthalmus*) (Thy *et al*., 2017), snakehead (*Channa striata*) (Talpur *et al.,* 2014), white carp (*Cirrhinus mrigala*) (Bhatnagar and Lamba, 2017), common carp (*C.carpio*) (Mohammadian *et al*., 2022) and Japanese eel (*Anguilla japonica*) (Lee *et al*., 2017).

Hematological variables are considered prognostic indicators and vital diagnostic tools for the assessment of fish health, reflecting the physiological and pathological responses in fish (Mohammadian *et al*., 2022). Similarly to our findings, improvement of hematological indices using dietary supplementation with *Bacillus* spp. has been demonstrated in Asian seabass (Adoraian *et al*, 2019; Ghanei Motlagh *et al*., 2021; Mozanzadeh *et al*., 2023), red seabream (Zaineldin *et al*, 2018), Nile tilapia (Hassaan *et al*, 2014; Abdel-Aziz *et al*, 2020), African catfish (Reda *et al*, 2018), Shabot (*Tor grypus)* (Mohammadian *et al*., 2019a) and Rohu (Mukherjee *et al*, 2019). It has been reported that probiotics could promote hematopoiesis which may be associated with diminished content of blood cortisol, provided essential micronutrients particularly B-Group vitamins and positively modulated gut microbiota (Carneveli *et al*, 2006, Mohapatra *et al*, 2013, Mohapatra *et al*, 2014; Elshaghabee *et al*, 2017). Probiotics can also interact with the components of the immune system within the intestinal lumen, resulting in the stimulation of immune responses. In this study, TLC was significantly increased in WQQ group for 30 days (Table 2). Our results are in line with previous reports, suggesting that *lactobacillus* spp. can modulate innate immune responses in fish (Zaineldin *et al*, 2018; Mohammadian *et al*, 2019; Mozanzaeh *et al*., 2023). A higher level of Hb was found in the combined group in 30 days post-feeding when compared to the control group. It was in agreement with the findings of Olayinka and Afolabi (2013) reported the association between Hb concentration and fish activity. Taking this finding, higher activity was observed in the probiotic-fed fish throughout the whole experiment than the control group. There was no significant differences between TEC and Hct levels (days 30) in the control and probiotic-fed groups during the experiment. This finding was in agreement with the results of previous works of Capkin and Altinok (2009). The probiotics indicate the increase of some blood parameters as a result of hemopoietic stimulation. Overall, our results suggest that the fish that received QQ and WQQ probiotics had healthier conditions compared to non-probiotic groups and, enhanced the host tolerance to possible stressors.

The modulatory effects of probiotics on the antioxidant defense system of fish have been demonstrated in previous studies (Mohammadian *et al*., 2022; Mozanzadeh *et al*., 2023). In the present research, higher hepatic activities of CAT and SOD were recorded in common carp fed with dietary QQ2 for 30 and 60 days, while higher hepatic activities of GSH were recorded in

common carp fed with dietary QQ1 for 30 and 60 days. In the current study, the malondialdehyde (MDA) analysis of fish fed with the QQ1+QQ2 (group 4) diets increased remarkably compared to the other groups only at day 60. In this regard, feeding gibel carp with an AHL-degrading probiotic (*B. frmus* sw40) led to an increased level of serum SOD but reduced contents of serum MDA and GSH (Li *et al*., 2019). The elevated activities of serum SOD, CAT and GSH as well as the decreased level of MDA were observed in Pengze crucian carp (*Carassius auratus* var. Pengze) fed with *B. cereus* (Yang *et al*., 2020). Moreover, dietary supplementation with *B. cereus* 39HN resulted in the highest levels of serum SOD in African catfish (*Clarias gariepinus*) (Reda *et al*., 2018). These protective effects of dietary probiotics on prevention of oxidative stress may be attributed to their potential for direct scavenging active oxidants, production of enzymes and metabolites with antioxidant activity, positive modulation of intestinal microbiota composition, biosynthesis of essential micronutrients with antioxidant activity such as vitamins B12 and B6, increased bioavailability of dietary antioxidants from the gut and regulation of antioxidant-associated signaling pathways in host (Wang *et al*., 2017 ; Hoseinpouri Ghasemabad Sofla¸ *et al*., 2024). SOD, CAT, GSH and MDA are among the most reliable markers associated with oxidative stress response. SOD and CAT are two essential components of the antioxidant enzyme system catalyzing the dismutation of the superoxide anion radical to hydrogen peroxide and water, and the decomposition of hydrogen peroxide into oxygen and water, respectively (Peixoto *et al*., 2016). GSH is a non-enzymatic antioxidant with a high abundance of sulfhydryl (thiol) groups that plays a key role in maintaining of intracellular oxidoreductive balance through scavenging reactive oxygen species (such as hydrogen peroxide in conjunction with glutathione peroxidase GPx) and detoxifcation of organic hydroperoxides (Zengin and Yilmaz, 2016). The reduction in hepatic GSH is associated with depletion during oxidative stress, decreased synthesis and increased utilization (Lu, 2020). MDA is one of the end products of lipid peroxidation and is frequently used as a biomarker of cell membrane injury. In this study, elevated activities of CAT and SOD, reduced contents of GSH, and increased levels of MDA were observed in QQ1+QQ2 and control groups compared to the other groups, especially at day 60. The mix QQ probiotics (QQ1+QQ2) in this study resulted in higher MDA levels in the liver as well as lower SOD activity in the liver that probably supported the worst antagonistic effect of two QQ bacteria compared to single use.

The results of the present study also demonstrate that the level of mortality in fish fed with the diets supplemented by *C. freundii* (QQ1) and *B. foraminis* (QQ2) decreased by 30.6 compared to the control group. However, no clear mechanism has been suggested for this protection and for the difference in protectiveness between QQ bacteria. A possible explanation may be differences in the probiotic's capabilities to modulate physiological responses in fish. *L. plantarum*, included as a without

characteristics QQ, caused a 40 % decrease in the level of mortality seen in the probiotic-fed fish compared to the control group (60.3 %), but did not give better protection compared to the experimental QQ isolated from the intestine of *C. carpio*. Our results were similar to the findings of Takafoyan *et al*. (2024), Chen *et al*. (2020a, 2020b), Torabi Delshad *et al*. (2019), Ghanei-Motlagh *et al*. (2019), Bhatnagar and Lamba (2017) and Bhatnagar and Aly *et al*. (2008), Giri *et al*. (2013), who recorded higher survival rate in fish fed with a diet containing probiotics compared with those fed with normal diet. Dietary supplementation with defined probiotics may be an effective biotherapeutic or prophylactic means in aquaculture (Mohammadain *et al*., 2016). Our results showed that *C. carpio* fed with the probiotic isolated from *C. carpio* intestine raised the survival rate when challenged with *A. hydrophilla*, suggesting that feeding with the probiotics for eight or more weeks is effective in helping to control Aeromonas infections in fish. There are many reports relating to enhanced protection against various diseases by feeding probiotic-containing food. The effects of probiotic bacteria have been studied in relation to the fish species and their genetic makeup, the feeding lengths, dosage and the type and origin of probiotic strain (Takafoyan *et al*., 2024). These results collectively suggest that dietary supplementation of gut QQ of *C. carpio* in form mix (QQ1+QQ2) not only improved their immune responses as well as the common probiotics like *Lactobacillus* bacteria but also in some respects is better. The observed improvement in the immune parameters measured here as a result of feeding the probiotic bacteria may be useful indicators for screening new strains of probiotic bacteria for use in aquaculture. When selecting a potential probiotic both its safety and its efficacy should be considered, both of the experimental species used here were originally isolated from the GI tract of *C. carpio* and are therefore autochthonous to the species and environment, and these probiotics could be useful because they are safety. The probiotic bacteria have to be administered at an optimal dose depending on the size and species of fish and probiotic strain used; thus, further investigation into the effectiveness of these probiotics is warranted.

Conclusion

The results obtained in this study suggest that, after full validation of their efficacy in the field and safety considerations, the application of QQ bacteria with high probiotic potential could be developed commercially as novel dietary supplements for control of infections caused by *Aeromonas* pathogens in the aquaculture industry. Moreover, the characteristic of spore formation by a certain QQ probiotic (*Bacillus* spp.) protects these kinds of strains from pressure and heat during the manufacturing processes, which simplifies their incorporation into commercial pellets.

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Conflicts of interest

The authors declare that they have no conflict of interest.

References

Abarike, E.D., Cai, J., Lu, Y., Yu, H., Chen, L., Jian, J., Tang, J., Jun, L. and Kuebutornye, F.K., 2018. Effects of a commercial probiotic BS containing *Bacillus subtilis* and *Bacillus licheniformis* on growth, immune response and disease resistance in Nile tilapia, *Oreochromis niloticus*. *Fish and Shellfsh Immunology*, 82, 229–238. DOI:10.1016/j.fsi.2018.08.037

Abdel-Aziz, M., Bessat, M., Fadel, A. and Elblehi, S., 2020. Responses of dietary supplementation of probiotic effective microorganisms (EMs) in Oreochromis niloticus on growth, hematological, intestinal histopathological, and antiparasitic activities. *Aquaculture International*, 28, 947-963. DOI:10.1007/s10499-019-00505-z

Adorian, T.J., Jamali, H., Farsani, H.G., Darvishi, P., Hasanpour, S., Bagheri, T. and 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, 248-255. DOI:10.1007/s12602-018-9393-z

Alishahi, M., Shirali, T., Tabandeh, M.R. and Ghorbanpour, M., 2022. Influence of p-coumaric acid, as a medicinal plant phenolic compound, on expression of virulence genes and pathogenicity of *Aeromonas hydrophila* in common carp. *Aquaculture International*, 30(**6**), 2997-3016. DOI:10.1007/s10499-022-00947-y

Aly, S.M., Abdel-Galil Ahmed, Y., Abdel-Aziz Ghareeb, A. and Mohamed, M.F., 2008. 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 and Shellfish Immunology,* 25, 128-136. DOI:10.1016/j.fsi.2008.03.013

Bhatnagar, A. and Lamba, R., 2017. Molecular characterization and dosage application of autochthonous potential probiotic bacteria in *Cirrhinus mrigala*. *Journal of FisheriesSciences com*. 11(**2**), 46. DOI:10.21767/1307-234X.1000117

Bhatnagar, A. and Saluja, S., 2019. Synergistic effects of autochthonous probiotic bacterium and Mentha piperita diets in *Catla catla* (Hamilton, 1822) for enhanced growth and immune response. *Fisheries and Aquatic Science*. 22, 16 29. DOI:10.1186/s41240-019-0130-7

Budi˜ no, B., Cal, R.M., Piazzon, M.C. and Lamas, J., 2006. The activity of several components of the innate immune system in diploid and triploid turbot. *Comparative Biochemistry and Physiology Part A*. 145(**1**), 108–113. DOI:10.1016/j.cbpa.2006.05.007

Capkin, E. and Altinok, I., 2009. Effects of dietary probiotic supplementations on prevention/treatment of yersiniosis disease. *journal Applied Microbiology*. DOI:106, 1147-1153.

Carnevali, O., de Vivo, L., Sulpizio, R., Gioacchini, G., Olivotto, I., Silvi, S. and Cresci, A., 2006. Growth improvement by probiotic in European sea bass juveniles (*Dicentrarchus labrax*, L.), with particular attention to IGF-1, myostatin and cortisol gene expression. *Aquaculture*, 258(**1-4**), 430-438. 10.1007/s12602-019-09552-7 . DOI:10.1016/j.aquaculture.2006.04.025

Case, R. J., Labbate, M., and Kjelleberg, S., 2008. AHL-driven quorum-sensing circuits: their frequency and function among the Proteobacteria. *Multidisciplinary of Microbial Ecology journal*, *2*(4), 345-349. DOI: 10.1038/ismej.2008.13

Chen, J., Lu, Y., Ye, X., Emam, M., Zhang, H. and Wang, H., 2020a. Current advances in Vibrio harveyi quorum sensing as drug discovery targets. *European Journal of Medicinal Chemistry*. 207, 112741. DOI:10.1016/j.ejmech.2020.112741

Chen, B., Peng, M., Tong, W., Zhang, Q. and Song, Z., 2020b. The quorum quenching bacterium *Bacillus licheniformis* T-1 protects zebrafsh against *Aeromonas hydrophila* infection. Probiotics Antimicrobial *Proteins*. 12(**1**), DOI: 160-171. 0.1007/s12602-018-9495-7.

Chu, W., Lu, F., Zhu, W. and Kang, C., 2011. Isolation and characterization of new potential probiotic bacteria based on quorum-sensing system. *journal Apply Microbiology*. 110(**1**), 202-208. DOI:10.1111/j.1365-2672.2010.04872.x

Dawood, M.A., Magouz, F.I., Salem, M.F., Elbialy, Z.I. and Abdel-Daim, H.A., 2019. Synergetic effects of *Lactobacillus plantarum* and β-glucan on digestive enzyme activity, intestinal morphology, growth, fatty acid, and glucose-related gene expression of genetically improved farmed tilapia. *Probiotics and Antimicrobial Proteins*. 12, 389–399. DOI: 10.1007/s12602-019-09552-7

Drabkin, D. L., 1950. The distribution of the chromoproteins, hemoglobin, myoglobin, and cytochrome c, in the tissues of different species, and the relationship of the total content of each chromoprotein to body mass. *Journal of Biological Chemistry*, *182*(1), 317-333.

Ellis, A.E., 1990. Serum antiproteases in fish and lysozyme assays. In: Stolen JS, Fletcher TC, Anderson DP, Roberson BS, Van Muiswinkel WB (eds) Techniques in Fish Immunology. SOSPublications, Fair Haven, NJ, pp. 95–103.

Ellman, G.L., 1959. Tissue sulfhydryl groups. *Archives of Biochemistry and Biophysics* , 82(**1**), 70–77. DOI:10.1016/0003-9861(59)90090-6

Elshaghabee, F.M., Rokana, N., Gulhane, R.D., Sharma, C. and Panwar, H., 2017. *Bacillus* as potential probiotics: status, concerns, and future perspectives. Frontiers in Microbiology, 8, 1490.

FAO. (2016). The State of World Fisheries and Aquaculture. 200 pp.

Galloway, W. R., Hodgkinson, J. T., Bowden, S. D., Welch, M. and Spring, D. R., 2011. Quorum sensing in Gram-negative bacteria: small-molecule modulation of AHL and AI-2 quorum sensing pathways. *Chemical Reviews*, 111(**1**), 28-67. DOI:10.1021/cr100109t

Gatesoupe, F., 1999. The use of probiotics in aquaculture. *Aquaculture,* 180, 147-165. DOI:10.1016/S0044-8486(99)00187-8

Ghanei-Motlagh, R., Mohammadian, T., Gharibi, D., Menanteau-Ledouble, S., Mahmoudi, E., Khosravi, M., ... and El-Matbouli, M., 2019. Quorum quenching properties and probiotic potentials of intestinal associated bacteria in Asian sea bass Lates calcarifer. *Marine Drugs*, 18(**1**), 23. DOI:10.3390/md18010023

Ghanei-Motlagh R, Mohammadian T, Gharibi D, Khosravi M, Mahmoudi E, Zarea M, and Menanteau-Ledouble,

S., 2021. Feed supplementation with quorum quenching probiotics with anti-virulence potential improved innate immune responses, antioxidant capacity and disease resistance in Asian seabass (*Lates calcarifer*). *Aquaculture*, 535, 736345. DOI:10.1016/j.aquaculture.2020.735874

Giri, S.S., Sukumaran, V. and Oviya, M., 2013. Potential probiotic *Lactobacillus plantarum* VSG3 improves thegrowth, immunity, and disease resistance of tropical freshwater fish, *Labeo rohita*. *Fish and Shellfsh Immunol*ogy. 34(**2**), 660–666. DOI:10.1016/j.fsi.2012.12.008

Harikrishnan, R., Kim, M.C., Kim, J.S., Balasundaram, C. and Heo, M.S., 2011. Probiotics and herbal mixtures enhance the growth, blood constituents, and nonspecifc immune response in *Paralichthys olivaceus* against S*treptococcus parauberis*. *Fish and Shellfsh Immunol*ogy. 31 (**2**), 310–317. DOI:10.1016/j.fsi.2011.05.020

Hassaan, M.S., Soltan, M.A. and Ghonemy, M.M.R., 2014. Effect of synbiotics between *Bacillus licheniformis* and yeast extract on growth, hematological and biochemical indices of the Nile tilapia (*Oreochromis niloticus*). *Egyptian Journal of Aquatic Research*, 40(**2**), 199-208. DOI:10.1016/j.ejar.2014.04.001

Hoseinpouri Ghasemabad Sofla, M., Soltani, M., Mohammadian, T. and Shamsaie Mehrgan, M., 2024. Immunological, oxidative stress, and biochemical responses of Salmo trutta caspius orally subjected to Bacillus probiotics (*Bacillus subtilis* and *B. licheniformis*) and sodium diformate. *Iranian Journal of Fisheries Sciences*, 23(**1**), 85-108. DOI:10.22092/ijfs.2024.130826.

Irianto, A. and Austin, B., 2002. Use of probiotics to control furunculosis in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Diseases,* 25, 333-342. DOI:10.1046/j.1365-2761.2002.00375.x

Jiang, Y., Zhou, S. and Chu, W., 2019. The effects of dietary *Bacillus cereus* QSI-1 on skin mucus proteins profle and immune response in crucian carp (*Carassius auratus gibelio*). *Fish and Shellfsh Immunol*ogy. 89, 319–325. DOI:10.1016/j.fsi.2019.04.014.

Kerr, M.G., 2008. Veterinary laboratory medicine: Clinical biochemistry and haematology. Second edition. John Wiley and Sons. New York. 355 P.

Kim, D. H. and Kim, D. Y., 2013. Microbial diversity in the intestine of olive flounder (*Paralichthys olivaceus*). *Aquaculture*, *414*, 103-108. DOI:10.1016/j.aquaculture.2013.06.008

Koroliuk, M.A., Ivanova, L.I., Maĭorova, I.G. and Tokarev, V.E., 1988. Metod opredeleniia aktivnosti katalazy [A method of determining catalase activity]. *Laboratornoe Delo,* 1, 16-9.

Lee, S., Katya, K., Park, Y., Won, S., Seong, M., Hamidoghli, A. and Bai, S.C., 2017. Comparative evaluation of dietary probiotics *Bacillus subtilis* WB60 and *Lactobacillus plantarum* KCTC3928 on the growth performance, immunological parameters, gut morphology and disease resistance in Japanese eel, *Anguilla japonica*. *Fish and Shellfsh Immunol*ogy. 61, 201 210. DOI:10.1016/j.fsi.2016.12.035.

Li, M.Y., Xi, B.W., Qin, T., Chen, K., Ren, M.C. and Xie, J., 2019. Indigenous AHL-degrading bacterium *Bacillus frmus* sw40 affects virulence of

pathogenic *Aeromonas hydrophila* and disease resistance of gibel carp. *Aquaculture. Res*earch. 50(**12**), 3755-3762. DOI:10.1111/are.14338

Lu, S.C., 2020. Dysregulation of glutathione synthesis in liver disease. *Liver Research*. 4 (**2**), 64–73. DOI:10.1016/j.livres.2020.05.003

Magnadóttir, B., 2006. Innate immunity of fish (overview). *Fish and shellfish immunology*, *20*(2), 137-151. DOI: 10.1016/j.fsi.2004.09.006

Makled, S.O., Hamdan, A.M. and El-Sayed, A.F.M., 2019. Growth promotion and immune stimulation in Nile Tilapia, *Oreochromis niloticus*, fngerlings following dietary administration of a novel marine probiotic, *Psychrobacter maritimus* S. *Probiotics and Antimicrobial Proteins*. 12, 365–374. DOI:10.1007/s12602-019-09575-0

Meidong, R., Khotchanalekha, K., Doolgindachbaporn, S., Nagasawa, T., Nakao, M., Sakai, K. and Tongpim, S., 2018. Evaluation of probiotic *Bacillus aerius* B81e isolated from healthy hybrid catfsh on growth, disease resistance and innate immunity of Pla-mong *Pangasius bocourti*. *Fish and Shellfsh Immuno*logy. 73, 1–10. DOI:10.1016/j.fsi.2017.11.032

Mihara, M. and Uchiyama, M., 1978. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. *Analytical Biochemistry*, 86, 271-278. DOI:10.1016/0003-2697(78)90342-1

Mohammadian, T., Alishahi, M., Tabandeh, M. R., Jangaran Nejad, A., Karami, E. and Zarea, M. (2019). Effects of autochthonous probiotics, isolated from *Tor grypus* (Karaman, 1971) intestine and Lactobacillus casei (PTCC 1608) on expression of immune-related genes. *Aquaculture International*, *27*, 239-260. DOI:10.1007/s10499-018-0320-9

Mohammadian, T., Alishahi, M., Tabandeh, M.R., Ghorbanpoor, M., Gharibi, D. and Tollabi, M., 2016. Probiotic effects of *Lactobacillus plantarum* and *L. delbrueckii* ssp. bulguricus on some immune-related parameters in Barbus grypus. *Aquaculture International*, 24, 225-42. 24(**1**), 225-242. DOI:10.1007/s10499-015-9921-8

Mohammadian, T., Alishahi, M., Tabandeh, M. R., Jangaran Nejad, A., Karami, E. and Zarea, M., 2019. Effects of autochthonous probiotics, isolated from *Tor grypus* (Karaman, 1971) intestine and *Lactobacillus casei* (PTCC 1608) on expression of immune-related genes. *Aquaculture International*, 27, 239-260. DOI:10.1007/s10499-018-0320-9

Mohammadian, T., Monjezi, N., Peyghan, R. and Mohammadian, B., 2022. Effects of dietary probiotic supplements on growth, digestive enzymes activity, intestinal histomorphology and innate immunity of common carp (*Cyprinus carpio*), a field study. *Aquaculture*, 549, 737787. DOI:10.1016/j.aquaculture.2021.737787

Mohapatra, S., Chakraborty, T., Kumar, V., DeBoeck, G. and Mohanta, K.N., 2013. Aquaculture and stress management: a review of probiotic intervention. *The Journal of Animal Physiology and Animal Nutrition*. 97(**3**), 405-430. DOI:10.1111/j.1439-0396.2012.01301.x

Mohapatra, S., Chakraborty, T., Prusty, A.K., PaniPrasad, K. and Mohanta,

K.N., 2014. Beneficial effects of dietary probiotics mixture on hemato-immunology and cell apoptosis of *Labeo rohita* fingerlings reared at higher water temperatures. *PloSone*, 9(**6**). DOI:10.1371/journal.pone.0100929

Mozanzadeh, M.T., Mohammadian, T., Ahangarzadeh, M., Houshmandm H, and Osroosh, E., 2023. Feeding Strategies with Multi-Strain Probiotics Affect Growth, Health Condition, and Disease Resistance in Asian Seabass (*Lates calcarifer*). *Probiotics and Antimicrobial Proteins*, 1-19. DOI:10.1007/s12602-023-10207-x

Mukherjee, A., Chandra, G. and Ghosh, K., 2019. Single or conjoint application of autochthonous *Bacillus* strains as potential probiotics: Effects on growth, feed utilization, immunity and disease resistance in Rohu, *Labeo rohita* (Hamilton). *Aquaculture*, 512, 734302. DOI:10.1016/j.aquaculture.2019.734302

Mzula, A., Wambura, P. N., Mdegela, R. H. and Shirima, G. M., 2019. Phenotypic and molecular detection of *Aeromonas* infection in farmed Nile tilapia in southern highland and northern Tanzania. *Heliyon*, 5(**8**). DOI:10.1016/j.heliyon.2019.e02220

Nain, Z., Adhikari, U. K., Abdulla, F., Hossain, N., Barman, N. C., Mansur, F. J., ...and Karim, M. M., 2020. Computational prediction of active sites and ligands in different AHL quorum quenching lactonases and acylases. *Journal of Biosciences*, 45, 1-19.

Nakanishi, T., Hikima, J.I. and Yada, T., 2018. Osteichthyes: Immune systems of teleosts (Actinopterygii). In: Cooper, E. (Ed.), In Advances in Comparative Immunology. Springer Cham, Switzerland. pp 687-749. DOI:10.1007/978-3-319-76768-019

Nayak, S.K., 2010. Probiotics and immunity: A fish perspective. *Fish and Shellfsh Immunol*ogy, 29, 2–14. DOI:10.1016/j.fsi.2010.02.017

Newaj-Fyzul, A., Adesiyun, A. A., Mutani, A., Ramsubhag, A., Brunt, J. and Austin, B., 2007. *Bacillus subtilis* AB1 controls Aeromonas infection in rainbow trout (*Oncorhynchus mykiss*, Walbaum). *Journal of Applied Microbiology*, *103*(5), 1699-1706. DOI: 10.1111/j.1365-2672.2007.03402.x

Nikoskelainen, S., Ouwehand, A., Salminen, S. and Bylund, G., 2001. Protection of rainbow trout (*Oncorhynchus mykiss*) from furunculosis by *Lactobacillus rhamnosus*. *Aquaculture,* 198, 229-236. DOI:10.1016/S0044-8486(01)00593-2

Olayinka, A.S. and Afolabi, O.O., 2013. Evaluation of the effects of *Lactobacillus acidophilus* on the haematological parameters of *Clarias gariepinus*. *Internationa Journal Research Fish Aquaculture*, 3, 38-41. DOI:10.1111/j.1365-109.2009.02265.x

Palma, M. and Viegas, I., 2022 Aquaculture: farming our food in water. In: Leal Filho, W., Azul, A.M., Brandli, L., Lange Salvia, A. and Wall, T. (eds.) Life Below Water. Cham: Springer International Publishing. pp. 44-52. DOI: 10.1007/978-3-319-98536-7_2

Peixoto, M.J., Salas-Leit´ on, E., Pereira, L.F., Queiroz, A., Magalh˜ aes, F., Pereira, R., Abreu, H., Reis, P.A., Gonçalves, J.F.M. and de Almeida Oz´ orio, R.O., 2016. Role of dietary seaweed supplementation on growth performance, digestive capacity and immune and stress responsiveness in European seabass (*Dicentrarchus*

labrax). *Aquaculture Reports,* 3, 189 197. DOI:10.1016/j.aqrep.2016.03.005

Pereira, G., Pereira, S.A., Soares, A., Mouri˜ no, J.L.P. and Merrifeld, D., 2019. Autochthonous probiotic bacteria modulate intestinal microbiota of Pirarucu, *Arapaima gigas*. *Journal of the World Aquaculture Society*, 50, 1152 1167. DOI:10.1111/jwas.12638

Reda, R.M., El-Hady, M.A., Selim, K.M. and 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 and Shellfsh Immunol*ogy, 80, 416-425. DOI:10.1016/j.fsi.2018.06.031

Reed, L.V. and Muench, H., 1938. A simple method of estimating fifty percent end points. *The American Journal of Tropical Medicine and Hygiene*, 27, 493–497.

Sareyyupoglu, B., Cantekin, Z. and Mustak, H.K., 2010. Investigation of Brucella antibodies in bovine by rose Bengal plate test (RBPT), serum agglutination test (SAT), microagglutination test (MAT) and 2-mercaptoethanolmicroagglutination (2-MEMAT) test. *Ankara Üniversitesi Veteriner Fakültesi Dergisi*, 57(**15**), 157-160. DOI:10.1501/Vetfak_0000002368

Shastry, R.P., Dolan, S.K., Abdelhamid, Y., Vittal, R.R. and Welch, M., 2018. Purification and characterisation of a quorum quenching AHL-lactonase from the endophytic bacterium *Enterobacter* sp. CS66. *FEMS Microbiology Letters*, 365(**9**), fny054. DOI:10.1093/femsle/fny054

Shefat, S.H.T., 2018. Use of probiotics in shrimp aquaculture in Bangladesh. *Acta Scientific Microbiology*, 1(**11**), 20-27.

Takafouyan, M., Mohammadian, B., Mohammadian, T. and Mesbah, M., 2024. Autochthonous probiotic in Asian sea bass (*Lates calcarifer*) diet: reduces excessive liver lipid deposition and resistance against Streptococcus iniae infection. *Iranian Journal of Fisheries Sciences*, 23(**4**), 669-683.

Talpur, A.D., Munir, M.B., Mary, A. and Hashim, R., 2014. Dietary probiotics and prebiotics improved food acceptability, growth performance, haematology and immunological parameters and disease resistance against *Aeromonas hydrophila* in snakehead (*Channa striata*) fngerlings. *Aquaculture*, 426, 14–20. DOI:10.1016/j.aquaculture.2014.01.013

Thy, H.T.T., Tri, N.N., Quy, O.M., Fotedar, R., Kannika, K., Unajak, S. and Areechon, N., 2017. Effects of the dietary supplementation of mixed probiotic spores of *Bacillus amyloliquefaciens* 54A, and *Bacillus pumilus* 47B on growth, innate immunity and stress responses of striped catfsh (*Pangasianodon hypophthalmus*). *Fish and Shellfsh Immunol*ogy, 60, 391–399. DOI:10.1016/j.fsi.2016.11.016

Torabi Delshad, S., Soltanian, S., Sharifiyazdi, H. and Bossier, P., 2019. Effect of catecholamine stress hormones (dopamine and norepinephrine) on growth, swimming motility, biofilm formation and virulence factors of *Yersinia ruckeri in vitro* and an *in vivo* evaluation in rainbow trout. *Journal of Fish Disease*, 42(**4**), 477-487. DOI:10.1111/jfd.12934

Van Kessel, M.A., Mesman, R.J., Arshad, A., Metz, J.R., Spanings, F.T., van Dalen, S.C., and Op den Camp, H.J., 2016. Branchial nitrogen cycle symbionts can remove ammonia in

fish gills. *Environmental Microbiology Reports*, 8(**5**), 590-594. DOI:10.1111/1758-2229.12407

Wang, Y., Wu, Y., Wang, Y., Xu, H., Mei, X., Yu, D., Wang, Y. and Li, W., 2017. Antioxidant properties of probiotic bacteria. *Nutrients*, 9(**5**), 521. DOI:10.3390/nu9050521

Winterbourn, C.C. and Kettle, A.J., 2013. Redox reactions and microbial killing in the neutrophil phagosome. Antioxid. *Antioxidants & Redox Signaling*, 18 (**6**), 642–660. DOI:10.1089/ars.2012.4827

Xavier, K.B. and Bassler, B.L., 2005. Interference with AI-2-mediated bacterial cell–cell communication. *Nature*, 437(**7059**), 750-753. DOI:10.1038/nature03960

Yang, G., Shen, K., Yu, R., Wu, Q., Yan, Q., Chen, W., Ding, L., Kumar, V., Wen, C. and Peng, M., 2020. Probiotic (*Bacillus cereus*) enhanced growth of Pengze crucian carp by modulating the antioxidant defense response and exerting benefcial impacts on infammatory response via Nrf2 activation. *Aquaculture*, 529, 735691. DOI:10.1016/j.aquaculture.2020.735691

Zaineldin, A.I., Hegazi, S., Koshio, S., Ishikawa, M., Bakr, A., El-Keredy, A.M., Dawood, M.A., Dossou, S., Wang, W. and Yukun, Z., 2018. *Bacillus subtilis* as probiotic candidate for red sea bream: growth performance, oxidative status, and immune response traits. *Fish and Shellfsh Immunol*ogy, 79, 303-312. DOI:10.1016/j.fsi.2018.05.035

Zengin, H., Yilmaz, Ö., Gokce, Z., and Demir, E., 2016. Antioxidant enzyme activities and some biochemical changes in rainbow trout *Oncorhynchus mykiss* (Walbaum 1792) Yolk-Sac Larvae. *Turkish Journal of Fisheries and Aquatic Sciences*, 16, 961-970. DOI: 10.4194/1303-2712-v

Zhang, C.N., Zhang, J.L., Guan, W.C., Zhang, X.F., Guan, S.H., Zeng, Q.H. and Cui, W., 2017. Effects of *Lactobacillus delbrueckii* on immune response, disease resistance against *Aeromonas hydrophila,* antioxidant capability and growth performance of C*yprinus carpio* Huanghe var. *Fish and Shellfsh Immunol*ogy, 68, 84–91. DOI:10.1016/j.fsi.2017.07.012