

Research Article

Efficacy of indigenous bacteria with quorum quencher properties on biochemical factors of Common carp (*Cyprinus carpio*)

M. A. Abdulaziz¹, T. Mohammadian^{2,5*}, M. Mesbah^{2,5}, D. Gharibi^{3,5}, S. M. Jalali⁴

¹Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran

²Department of Livestock, Poultry and Aquatic animal Health, Shahid Chamran University of Ahvaz, Ahvaz, Iran

³Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran

⁴Department of Clinical Science, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran

⁵Member of Excellence Center of Warm Water Fish Health, Shahid Chamran University of Ahvaz, Ahvaz, Iran

Received: September 2024

Accepted: November 2024

Abstract

In this study, efficacy of two main probiotics (*Citrobacter freundii* and *Bacillus foraminis*) with indigenous quorum quenching (QQ) isolated from the intestine of *Cyprinus carpio* was studied on haemato-innate immune responses, antioxidant capacity and disease resistance of this fish species. Juveniles of *C. carpio* (n=450, weighing 50±10 g) were randomly divided into 6 equal groups (with 3 replications) and were fed on diets containing 1×10⁹ cfu g⁻¹ of *C. freundii* (QQ1, G1), *B. foraminis* (QQ2, G2), *L. plantarum* (without characteristics QQ, WQQ, G3), QQ1 + QQ2 (G4), QQ1 + QQ2+WQQ (combine, G5), and a control group. *Corresponding author's email: T.mohammadian@scu.ac.ir

diet (without probiotic) for 60 continuous days.

Our results showed, alkaline phosphatase (ALP) levels increased in QQ2 at 30 days but it declined later, while AST and ALT activities varied among treatments. Serum triglycerides, Total protein, albumin, Glucose and urea were significantly different in the probiotic treatments compared control group ($p<0.05$). Overall, indigenous probiotics improved various health parameters and reduced side effects in treated fish.

Keywords: *C. carpio*, Intestine bacteria, Indigenous quorum quenching, Biochemical factors

Introduction

Aquaculture, and more specifically aquatic culture, faces several challenges in order to increase production yield while maintaining sustainability. The various disease outbreaks that have been continuously affecting the sector for almost three decades, together with the increasing demand for environmentally friendly aquaculture and the pressure from customers for safe and traceable products, are fundamentally modifying the culture practices of important fish. It is today well accepted that these challenges can only be faced through the development of better management practices together with technical innovations. Among the solutions currently considered, those relying on nutrition and intestinal health management can play a critical role. The application of probiotics in aquaculture has been developed in this context and is now widely applied in aquaculture as a complementary tool for management of disease. Moreover, probiotics have beneficial impacts on the host due to inhibition of the disorder condition (Gatesoupe, 1999), alteration of biochemical factor (Shefat, 2018). 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 sector, about 4.19 million tonnes belong to common carp (*Cyprinus carpio*) (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 region. 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 disease. FAO (Year??) has shown that the use of probiotics is one of the best ways to increase 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. 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.*, 2020). 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.

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 indigenous probiotics was tested for the first time. The present investigation was undertaken to evaluate the changes in various biochemical parameters of common carp after feeding them with QQ bacteria isolated from their intestine.

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 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 responds to exogenous AHLs with *N*-acyl side chains from C₄ to C₈ 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). 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* weighing 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 1×10^9 cfu 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 $25.9 \pm 1.2^\circ\text{C}$. 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)	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. Blood samples were withdrawn from the caudal vein of four fish per aquarium using a 2.5-ml syringe. One part of collected blood was dropped in heparinized microtube and the residue was subjected to centrifugation (3000g, 10 min, 4 °C) to separate serum. The sera were then frozen at -80 °C until use.

Serum liver enzyme parameters

Serum alkaline phosphatase (ALP), alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), lactate dehydrogenase (LDH) and calcium were kinetically measured using the standard diagnostic kits according to the manufacturer's instruction (Pars Azmoon Co., Tehran, Iran). Serum ALP was determined using the standard method recommended by Deutsche Gesellschaft

Für Klinische Chemie (DGKC) according to the liberated p-Nitrophenol resulted from ALP activity at alkaline pH (Faremi *et al.*, 2008). Serum LDH-P activity was estimated by DGKC recommendations based upon the use of pyruvate substrate (Agrawal *et al.*, 2016). Serum ALAT and ASAT were measured as suggested by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) (Lustig *et al.*, 1988). Using the tested kits, a change of 0.001 Abs units per min was equivalent to 2.76 U/l ALP, 2 U/l ALAT, 2 U/l ASAT and 16.67 U/l LDH-P activity. The kit expected ranges for the biochemical parameters were as follows: Total protein (0.5-15 g/dl), albumin (0.2-6 g/dl), glucose (5-400 mg/dl), total cholesterol (5-500 mg/dl), triglycerides (5-700 mg/dl), calcium (0.4-25 mg/dl), ALP (3-858 U/l), ALAT (4-300 U/l), ASAT (2-300 U/l) and LDH (5-3000 U/l).

Serum biochemical parameters

Total protein, albumin, glucose, triglycerides and calcium were photometrically measured using commercially available kits used according to the manufacturer's guidelines (Pars Azmoon Co., Tehran, Iran). Total protein and albumin were respectively determined by the biuret and the bromocresol green (BCG) reactions as described elsewhere (Dati *et al.*, 1996). The enzymatic colorimetric method, glucose oxidase-phenol amino phenazone (GOD-PAP), was used to assay the profile of serum glucose (Barham and Trinder, 1972). Serum total triglycerides were determined enzymatically using the cholesterol oxidase phenol 4-aminoantipyrine peroxidase (CHOD-PAP) and glycerol phosphate oxidase-p-aminophenazone (GPO-PAP) methods, respectively (Wang *et al.*, 2008). Calcium measurement was carried out by the Arsenazo III method resulting in violet colored Arsenazo III: calcium complex in a neutral pH range (Bauer, 1981). In order to calculate the above-mentioned parameters, the following equation was used: (Abs sample/Abs standard) $\times$ concentration of standard. Where the absorbance of each serum sample and the respective standards was read against the blank at the specific

wavelength and incubation time for each parameter.

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 general linear model was 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 $\pm$ SD.

Results

Serum liver enzyme parameters

The ALP was increased during the 30 days of treatment in QQ2 group, while its activity declined at the end of the experiment. The lowest amount ALP was determined in combine group on the 30th day of the experiment (Table 2). The highest amount ALP was determined in QQ1 group on the 60th day of the experiment. The AST increased in QQ2 and control groups during the 30 days of probiotic feeding and thereafter increased (except QQ2 and WQQ groups) until the end of the experiment. Its lowest activity

was found in QQ1 and WQQ groups on day 30 and 60, respectively (Table 3).

The amount of ALT was significantly elevated on the 30th day of the feeding in QQ1 and control groups ($p<0.05$). we found changes in ALT activity of QQ1 group, 60

days after the probiotic feeding. The highest activity of ALT were observed on day 60 past the feeding in combine and control groups.

Table 2. Liver enzymes in *C. Carpio* fed feed supplemented with different probiotics for 60 days.

Parameters	Groups	Day 30	Day 60
Alkaline phosphatase (ALP)	Q1	358.5±39.42 Bb	467.69±18.65 Aa
	Q2	533.01±11.2 Aa	175.98±8.25 C,b
	WQ	447.31±53.1 ABa	307.05±9.15 B,b
	Q1+Q2	412.9±14.72 ABa	371.49±14.3 B,b
	Q1+Q2+WQ	101.96±15.54 Ca	226.82±35.19 BCb
	Control	432.91±43.15 Aa	216.2±15.17 BCb
Aspartate aminotransferase (AST)	Q1	164.39±6.08Bb	216.53±13.4 ABa
	Q2	237.47±11.1A,a	205.52±6.11 AB,a
	WQ	217.4±28.1A,a	186.5±7.11 B,a
	Q1+Q2	199.37±21.8 AB,b	314.65±8.25 A,a
	Q1+Q2+WQ	182.35±32.8 AB,b	254.47±14.1 AB,a
	Control	219.3±23.6 A,b	309.4±17.12A,a
Alanine transaminase (ALT)	Q1	3.62±0.6 Aa	0.51±0.18 Cb
	Q2	0.52±0.03 Ca	0.46±0.171 C,a
	WQ	0.41±0.12 Cb	0.78±0.04 BC,a
	Q1+Q2	0.42±0.041 Ca	0.48±0.05 C,a
	Q1+Q2+WQ	0.46±1.35 Cb	0.93±0.19 B,a
	Control	1.66±0.33 Ba	1.8±0.15 Aa

*Different lowercase letters indicate statistically significant differences between each of the experimental groups at various sampling time points (row) ($p<0.05$). Different capital letters denote significant differences between the experimental groups at a specified time point (column) ($p<0.05$). Data were expressed as means±SEM (n = 9).

Serum biochemical parameters

Table 3 represents the effects of supplemented QQ bacteria foods on biochemical parameters of *C. carpio*. On the 30th day of the experiment, there was

no significant difference in the serum calcium levels of *C. carpio* in all supplemented probiotic groups compared to the control group ($p>0.05$). Also, there was a significant increase in the serum

Triglyceride levels of *C. carpio* in all supplemented probiotic groups compared to the control group at days 30 ($p<0.05$).

Table 3. Biochemical parameters in *C. carpio* fed either regular feed or feed supplemented with probiotics for 60 days.

Parameters	Groups	Day 30	Day 60
Total protein (mg/dl)	Q1	3.5±0.42 ^{Ba}	3.69±0.65 ^{Ba}
	Q2	4.01±0.22 ^{ABa}	3.98±0.25 ^{AB,a}
	WQ	4.31±1.11 ^{Aa}	4.05±0.15 ^{AB,a}
	Q1+Q2	4.9±0.72 ^{ABa}	4.49±0.3 ^{A,a}
	Q1+Q2+WQ	4.96±0.54 ^{Aa}	3.82±0.19 ^{ABb}
	Control	3.91±0.15 ^{Ba}	4.2±0.17 ^{Aa}
Albumin (mg/dl)	Q1	0.39±0.08 ^{A Ba}	0.53±0.04 ^{ABa}
	Q2	0.47±0.1 ^{A,a}	0.52±0.11 ^{AB,a}
	WQ	0.4±0.1 ^{AB,a}	0.5±0.11 ^{AB,a}
	Q1+Q2	0.37±0.08 ^{AB,b}	0.65±0.025 ^{A,a}
	Q1+Q2+WQ	0.35±0.08 ^{AB,a}	0.47±0.11 ^{B,a}
	Control	0.3±0.06 ^{B,a}	0.4±0.12 ^{B,a}
Urea (mg/dl)	Q1	11.62±1.6 ^{Ba}	15.28±3.18 ^{Aa}
	Q2	12.2±1.3 ^{Ba}	16.66±1.71 ^{A,a}
	WQ	14.1±1.12 ^{Aa}	15.76±4.54 ^{A,a}
	Q1+Q2	10.62±1.41 ^{Ab}	15.16±2.5 ^{A,a}
	Q1+Q2+WQ	12.66±1.35 ^{Aa}	14.6±3.49 ^{A,a}
	Control	10.66±1.33 ^{Bb}	14.4±3.15 ^{Aa}
Creatine phosphokinase (mg/dl)	Q1	199.4±33.3 ^{Bb}	525.84±21.1 ^{Ba}
	Q2	ND	393.53±51/31 ^{B,a}
	WQ	14±3.32 ^{C,b}	787.7±36.14 ^{A,a}
	Q1+Q2	12.7±4.41 ^{C,b}	420.43.2±14.15 ^{B,a}
	Q1+Q2+WQ	236.4±24.5 ^{A,a}	193.47±22.49 ^{C,b}
	Control	24.6±3.3 ^{C,b}	531.19±51.95 ^{B,a}
Calcium (mg/dl)	Q1	8.65±0.75 ^{Aa}	8.5±0.51 ^{Aa}
	Q2	8.1±0.94 ^{Aa}	7.4±0.57 ^{Aa}
	WQ	7.9±0.6 ^{Aa}	8.04±1.7 ^{Aa}
	Q1+Q2	7.56±0.68 ^{Aa}	7.8±1.35 ^{Aa}
	Q1+Q2+WQ	9.3±0.44 ^{Aa}	8.1±0.65 ^{Aa}
	Control	8.14±0.49 ^{Aa}	7.55±0.88 ^{Aa}

Table 3 (continued):

Parameters	Groups	Day 30	Day 60
Glucose (mg/dl)	Q1	112.5±2.47 ^{Ab}	159.17±23.56 ^{ABa}
	Q2	115.6±1.7 ^{Ab}	183.8±12.47 ^{Aa}
	WQ	103.25±5.37 ^{A,b}	141.2±5.49 ^{B,a}
	Q1+Q2	128.66±2.88 ^{A,a}	127.5±5.92 ^{B,a}
	Q1+Q2+WQ	127.5±4.03 ^{A,b}	188.6±20.2 ^{A,a}
	Control	107.25±1.31 ^{A,b}	158.25±6.65 ^{AB,a}
Triglyceride (mg/dl)	Q1	315.8±44.9 ^{Aa}	258.3±46.54 ^{Bb}
	Q2	273.6±12.52 ^{Aa}	234.0±14.73 ^{Ba}
	WQ	312.4±1.84 ^{Aa}	223.8±26.71 ^{Bb}
	Q1+Q2	296.4±15.8 ^{Aa}	334.2±20.93 ^{Aa}
	Q1+Q2+WQ	293.6±9.5 ^{Aa}	272.2±15.02 ^{Ba}
	Control	234.8±8.5 ^{Ba}	277.4±10.92 ^{Ba}

*Different lowercase letters indicate statistically significant differences between each of the experimental groups at various sampling time points (row) ($p<0.05$). Different capital letters denote significant differences between the experimental groups at a specified time point (column) ($p<0.05$). Data were expressed as means±SEM (n=9).

But, a remarkable increase was observed in the Triglyceride level in the QQ1+QQ2 group compared to the control group and the other probiotic treatment at days 60 ($p<0.05$). Moreover, a remarkable increase was observed in the total protein levels in the combine (Q1+Q2+WQ) and WQ groups compared to the control group at days 30 ($p<0.05$). At the end of the feeding experiment, there was significantly decreased in the blood total protein levels of *C. carpio* in supplemented QQ1 groups compare to the control group ($p>0.05$). Furthermore, the statistical analysis of results revealed that QQ2 significantly increased the albumin levels compared to

the control group at days 30 ($P<0.05$). But, a remarkable increase was observed in the albumin level in the QQ1+QQ2 group compared to the control group and the other probiotic treatment at days 60 ($p<0.05$). Urea level was significantly increased in fish fed with a diet containing WQQ compared to the control at days 30 ($p<0.05$). At the end of the feeding experiment, there was no significant difference in the blood urea levels of *C. carpio* in all supplemented probiotic groups compare to the control group ($p>0.05$). A remarkable decrease was observed in the creatine phosphokinase level in the WQQ and QQ1+QQ2 groups compared to the

control group at days 30 ($p>0.05$). creatine phosphokinase level was significantly decreased in the combine (QQ1+QQ2+WQQ) group compared to the control ($p<0.05$). Also, glucose level was significantly decreased in fish fed with a diet containing 0.1, 0.15, and 0.2 KDF and 0.15 CaDF compared to the control ($p<0.05$). At the 30 days of the feeding experiment, there was no significant difference in the blood glucose levels of *C. carpio* in all supplemented probiotic groups compare to the control group ($p>0.05$). There was a significant decrease ($p<0.05$) in the serum glucose of the WQQ and QQ1+QQ2 compared to the control group ($p<0.05$).

Discussion

It is clear that more emphasis in future studies should be placed on elucidating the extent of probiotic modulation of the gut microbiota of fish, either as a direct implantation of the probiotic population and/or via changes in the indigenous microbial populations, in order to be able to apply probiotic applications with greater efficacy. The present literature is heavily focused on the bacterial microbiota and considerably less information is available on indigenous bacteria. In particular QQ

characteristic and their influence on indigenous probiotic bacteria must be the subject of future studies.

Blood biochemistry is a useful tool for the evaluation of health status and provide information for diagnosis and prognosis of fish diseases and disorders (Sharifuzzaman *et al.*, 2014; Hoseinpouri Ghasemabad Sofla *et al.*, 2024). The data related to the serum biochemical profile are summarized in Table 3. Serum total protein, albumin remained affected by the probiotic supplements at days 30 in combine group. Against our result, no significant changes were reported in tilapia fed with dietary *Bacillus subtilis* (1-deoxynojirimycin) or *Enterococcus faecium* (Wang *et al.*, 2008; Tang *et al.*, 2017). But similar, enhancement in the levels of serum proteins has been regarded as potentiated immune response in fish (Zhang *et al.*, 2013). Total protein was significantly higher in the combine group than the control group after infection ($p<0.05$). This might be due to the boosted immune responses fed with these probiont. Serum glucose content was significantly lower in fish fed with *L. plantarum* (WQQ) and QQ1+QQ2 compared to the other groups at the end of excrement ($p<0.05$). Several publications have suggested that probiotics particularly

Lactobacillus and *Bifidobacterium* spp. can reduce blood glucose (Falcinelli Zhang *et al.*, 2013, 2016, 2018). Elevated serum glucose in the QQ2 group and combine group could be related to higher α -amylase activity in this group, stored as glycogen in liver after transportation to blood circulation (Yang *et al.*, 2019). As stated by Mukherjee *et al.* (2019), increased glucose content might be due to hindered metabolic stress in fish fed with probiotic, however, further studies with respect to multifactorial-dependent metabolism of glucose in fish are needed to clarify the role of dietary probiotics in glucose homeostasis. The profile of serum triglycerides was significantly increment in all fish fed with probiotic in the probiotic groups compared to the control groups after at days 30 ($p < 0.05$). After 8 weeks feeding, serum triglycerides content was noticeably reduced in the probiotic groups relative to the QQ1+QQ2 groups ($p < 0.05$). The hypotriglyceridemic effects of probiotics have been previously attributed to positively modulated gut microbiota, increased short chain fatty acids in the gut and decreased transcription of several genes involved in triglyceride (*fit2* and *mgll*) metabolism (Dawood *et al.*, 2016; Falcinelli *et al.*, 2018). No significant alteration in serum calcium content was

observed ($p > 0.05$). Contrary to our findings, decrease in calcium content was observed in *Clarias gariepinus* infected with fish pathogens (Reda *et al.*, 2018).

According to our results, ALAT and ASAT are cytoplasmic enzymes commonly used to evaluate the liver cell membrane damage. However, ALAT is considered more specific for liver disease than ASAT (Arika *et al.*, 2016). Serum levels of ALP, ASAT, and ALAT showed significant differences among the treatments at days 30 and 60 ($p < 0.05$). Alteration of the hepatic enzymes following administration of tested probiotics may indicate that they had negative effect on the normal functions of liver. all the hepatic enzymes were markedly elevated in the control group compared to the probiotics groups ($p < 0.05$). The liver-type tissue non-specific alkaline phosphatase (TNAP) and intestinal alkaline phosphatase (IAP) are membrane-bound enzymes involved in protection against pathogen-associated molecular patterns (PAMPs) including flagellin and lipopolysaccharide (LPS) from gram-negative bacteria. In hepatic disorders, the accumulation of bioactive forms of lipopolysaccharide (LPS) in bile canaliculi and bile ducts induce further inflammation (Poupon, 2015). This might be associated with the higher levels of ALP in the some

probiotic groups and lower levels of ALP may be due to the protective effects of QQ probiotics used. The use of probiotics to reduce the levels of AST and ALT shows the potential of protecting the liver in the conducted researches. Always, investigating the levels of AST and ALT in the treatments that are done to improve fish health can show possible toxic effects related to the compounds used or interactions with other compounds in the environment. In the present study, liver enzymes across different treatments (with the exception of ALT) exhibited significant differences when compared to each other. This contrasts with findings from some other studies, where no significant differences in liver enzyme activity were observed between the probiotic groups and the control group. They found that the level of ALT and AST of tilapia fish serum was not affected by the diet containing the probiotic Biogen *B. subtilis*. In agreement to our study, the use of Biogen probiotic and surfactin, an antimicrobial lipopeptide produced by several strains of *B. subtilis*, showed that ALT and AST levels were significantly reduced in Nile tilapia (Zhai *et al.*, 2017). In another study, the activity of liver enzymes was investigated and they found that diets with probiotics increased

the serum level of AST (Beiwi and Al-Hisnawi, 2020). The difference between the results reported in the above studies can be due to the type of probiotics, the concentration of probiotics used, the species of fish studied, the administration time, the administration method, or the environmental conditions. Alkaline phosphatase (ALP) is a lysosomal enzyme that plays a role in the activation of macrophages and acts as an important antibacterial agent. Increased alkaline phosphatase activity is associated with increased enzyme production by macrophage cells. Alkaline phosphatase activity increased in the serum and mucus of large Indian carp (*Catla catla*) that was fed a diet containing *Bacillus subtilis* (Sangma and Kamilya, 2015). In another study, alkaline phosphatase activity increased in the serum and mucus of rainbow trout that were fed a diet supplemented with *Lactobacillus rhamnosus* (Andani *et al.*, 2012). Therefore, it can be concluded that the type of probiotics and consumption duration can reduce or increase liver damage and ultimately decrease or increase the activity level of these enzymes.

Conclusions

The results obtained in this study suggest that, after full validation of their efficacy in the field and safety considerations, application of QQ bacteria (*Bacillus* spp.) with high probiotic potential could be developed commercially as novel dietary supplements for Keeping the fish healthy.

Acknowledgments

This research was financed by a grant from the Shahid Chamran University of Ahvaz Research Council (Grant No. SCU.VP1401.153). The funding body had no role in the design of the study or interpretation of data.

Conflicts of interest

The authors declare that they have no conflict of interest.

References

Ahmad, M. B., Kulshreshtha, J. I. and Singh, G., 2011. Growth and pigment profile of *Spirulina platensis* isolated from Rajasthan, India. *Research Journal of Agricultural Sciences*, 22(1), 1, pp. 83-86.

Abarike, E.D., Cai, J., Lu, Y., Yu, H., Chen, L., Jian, J., Tang, J., Jun, L., 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 Shellfish Immunol.* 82, 229–238.

Abdel-Aziz, M., Bessat, M., Fadel, A., & 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.

Adorian TJ, Jamali H, Farsani HG, 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: 248-255. <https://doi.org/10.1007/s12602-018-9393-z>.

Agrawal, A., Gandhe, M.B., Gupta, D. and Reddy, M.V.R., 2016. Preliminary study on serum lactate dehydrogenase (LDH)-prognostic biomarker in carcinoma breast. *J. clin. diagn*, 10(3), BC06.

Alishahi, M., Shirali, T., Tabandeh, M. R., & 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.

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.

Andani, H., Tukmechi, A., Meshkini, S. and Sheikhzadeh, N., 2012. Antagonistic activity of two potential probiotic bacteria from fish intestines and investigation of their effects on growth performance and immune response in rainbow trout (*Oncorhynchus mykiss*). *Journal of Applied Ichthyology*, 28, 728-734.

Arika, W.M., Nyamai, D.W., Osano, K.O., Ngugi, M.P. and Njagi, E.N.M., 2016. Biochemical markers of in vivo hepatotoxicity. *J Clin Toxicol*, 6(2), 1-8.

Ashouri G et al (2018) Combined effects of dietary low molecular weight sodium alginate and *Pediococcus acidilactici* MA18/5M on growth performance, haematological and innate immune

responses of Asian sea bass (*Lateolabrax japonicus*) juveniles. *Fish Shellfish Immunol* 79:34–41

Beiwi D.A., Al-Hisnawi A. (2020). Effect of *Bacillus subtilis* as probiotic on intestinal microbiota and growth performance of common carp (*Cyprinus carpio*). In AIP Conference Proceedings. AIP Publishing LLC, 2290(1): 030004.

Bhatnagar, A., Lamba, R., 2017. Molecular characterization and dosage application of autochthonous potential probiotic bacteria in *Cirrhinus mrigala*. *J Fishscicom*. 11 (2), 46.

Bhatnagar, A., Saluja, S., 2019. Synergistic effects of autochthonous probiotic bacterium and *Mentha piperita* diets in *Catla catla* (Hamilton, 1822) for enhanced growth and immune response. *Fish Aquat. Sci.* 22, 16-29.

Budin, B., Cal, R.M., Piazzon, M.C., Lamas, J., 2006. The activity of several components of the innate immune system in diploid and triploid turbot. *Comp Biochem Physiol A Mol Integr Physiol*. 145 (1), 108–113.

Capkin, E., Altinok, I., (2009). Effects of dietary probiotic supplementations on

prevention/treatment of yersiniosis disease.

J. Appl. Microbiol. 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.

Chen, B., Peng, M., Tong, W., Zhang, Q., Song, Z., 2020b. The quorum quenching bacterium *Bacillus licheniformis* T-1 protects zebrafish against *Aeromonas hydrophila* infection. *Probiotics Antimicrob. Proteins*. 12 (1), 160-171.

Chen, J., Lu, Y., Ye, X., Emam, M., Zhang, H., Wang, H., 2020a. Current advances in *Vibrio harveyi* quorum sensing as drug discovery targets. *Eur. J. Med. Chem.* 207, 112741.

Chu, W., Lu, F., Zhu, W. and Kang, C., 2011. Isolation and characterization of new potential probiotic bacteria based on quorum-sensing system. *J. Appl. Microbiol*, 110(1), 202-208.

Dawood, M.A., Magouz, F.I., Salem, M.F., Elbialy, Z.I., 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. *Probiot. Antimicrob. Proteins*. 12, 389-399.

Ellis AE (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*. SOS Publications, Fair Haven, NJ, pp 95-103.

Ellman, G.L., 1959. Tissue sulfhydryl groups. *Arch. Biochem. Biophys.* 82 (1), 70-77.

Elshagabee, F.M., Rokana, N., Gulhane, R.D., Sharma, C. and Panwar, H., 2017. *Bacillus* as potential probiotics: status, concerns, and future perspectives. *Front Microbiol*, 8, 1490.

Fazio F (2019) Fish hematology analysis as an important tool of aquaculture: A review. *Aquaculture* 500:237-242

Galloway, W. R., Hodgkinson, J. T., Bowden, S. D., Welch, M., & 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.

Gatesoupe, F., 1999. The use of probiotics in aquaculture. *Aquaculture*, 180, 147-165.

Ghanei-Motlagh R, Mohammadian T, Gharibi D, Khosravi M, Mahmoudi E, Zarea M, *et al* (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.
<https://doi.org/10.1016/j.aquaculture.2020.735874>

Giri SS, Sukumaran V, Oviya M (2013) Potential probiotic *Lactobacillus plantarum* VSG3 improves the growth, immunity, and disease resistance of tropical freshwater fish, *Labeo rohita*. *Fish Shellfish Immunol* 34(2):660–666

Grant, K.R., 2015. Fish hematology and associated disorders. *Vet Clin North Am Exot Anim Pract*, 18(1), 83-103.

Harikrishnan, R., Kim, M.C., Kim, J.S., Balasundaram, C., Heo, M.S., 2011a. Probiotics and herbal mixtures enhance the growth, blood constituents, and nonspecific immune response in *Paralichthys olivaceus*

against *Streptococcus parauberis*. *Fish Shellfish Immunol*. 31 (2), 310–317.

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*). *Egypt J Aquat Res*, 40(2), 199-208.

Hoseinpouri Ghasemabad Sofla, M., Soltani, M., Mohammadian, T., & 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

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.

Jiang, Y., Zhou, S., Chu, W., 2019. The effects of dietary *Bacillus cereus* QSI-1 on skin mucus proteins profile and immune response in crucian carp (*Carassius auratus gibelio*). *Fish Shellfish Immunol*. 89, 319–325.

- Kerr, M.G., 2008. Veterinary laboratory medicine: Clinical biochemistry and haematology. Second edition. John Wiley and Sons. New York.
- Koroliuk MA, Ivanova LI, Maïorova IG, Tokarev VE (1988) Metod opredeleniia aktivnosti katalazy [A method of determining catalase activity]. Lab Delo 1:16-9. Russian. PMID: 2451064.
- Lee, S., Katya, K., Park, Y., Won, S., Seong, M., Hamidoghli, A., 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 Shellfish Immunol. 61, 201–210.
- Li, M.Y., Xi, B.W., Qin, T., Chen, K., Ren, M.C., Xie, J., 2019. Indigenous AHL-degrading bacterium *Bacillus firmus* sw40 affects virulence of pathogenic *Aeromonas hydrophila* and disease resistance of gibel carp. Aquac. Res. 50 (12), 3755–3762.
- Lu, S.C., 2020. Dysregulation of glutathione synthesis in liver disease. Liver Res. 4 (2), 64–73.
- Lustig, V., Papanastasiou-Diamandis, A. and Goldberg, D.M., 1988. Evaluation of commercially formulated aspartate aminotransferase and alanine aminotransferase activity determinations by the Scandinavian Committee on Enzymes and IFCC methods as modified for use with automated enzyme analysers. Clin. Biochem, 21(5), 283-290.
- Makled, S.O., Hamdan, A.M., El-Sayed, A.F.M., 2019. Growth promotion and immune stimulation in Nile Tilapia, *Oreochromis niloticus*, fingerlings following dietary administration of a novel marine probiotic, *Psychrobacter maritimus* S. Probiot. Antimicrob. Proteins. 12, 365–374.
- Meidong, R., Khotchanalekha, K., Doolgindachbaporn, S., Nagasawa, T., Nakao, M., Sakai, K., Tongpim, S., 2018. Evaluation of probiotic *Bacillus aerius* B81e isolated from healthy hybrid catfish on growth, disease resistance and innate immunity of Pla-mong *Pangasius bocourti*. Fish Shellfish Immunol. 73, 1–10.
- Mihara, M, Uchiyama, M 1978: Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal Biochem 86: 271-278.

Mohammadian, T., Alishahi, M., Tabandeh, M. R., Jangaran Nejad, A., Karami, E., & Zarea, M. (2019a). 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.

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.

Mohammadian, T., Monjezi, N., Peyghan, R., & 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.

Mohapatra, S., Chakraborty, T., Kumar, V., DeBoeck, G. and Mohanta, K.N., 2013. Aquaculture and stress management: a review of probiotic intervention. *J Anim Physiol Anim Nutr*, 97(3), 405-430.

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. *PloS one*, 9(6).

Mozanzadeh MT, Mohammadian T, Ahangarzadeh M, Houshmand H, et al (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. <https://doi.org/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.

Mzula, A., Wambura, P. N., Mdegela, R. H., & Shirima, G. M. (2019). Phenotypic and molecular detection of *Aeromonads* infection in farmed Nile tilapia in Southern highland and Northern Tanzania. *Heliyon*, 5(8).

- Nain, Z., Adhikari, U. K., Abdulla, F., Hossain, N., Barman, N. C., Mansur, F. J., ... & 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., Yada, T., 2018. Osteichthyes: Immune systems of teleosts (Actinopterygii). In: Cooper, E. (Ed.), In *Advances in Comparative Immunology*. Springer Cham, Switzerland, pp. 687-749.
- Nayak, S.K., 2010. Probiotics and immunity: a fish perspective. *Fish Shellfish Immunol.* 29, 2–14.
- 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.
- Olayinka, A.S., Afolabi, O.O., 2013. Evaluation of the effects of *Lactobacillus acidophilus* on the haematological parameters of *Clarias gariepinus*. *Int. J. Res. Fish. Aquacult.* 3, 38-41.
- Palma, M., Magnoni, L. J., Morais, S., & Viegas, I. (2023). Tributyrin supplementation in fish and crustacean nutrition: a review. *Reviews in Aquaculture*, 15(2), 785-800.
- Peixoto, M.J., Salas-Leitón, E., Pereira, L.F., Queiroz, A., Magalhães, F., Pereira, R., Abreu, H., Reis, P.A., Gonçalves, J.F.M., de Almeida Ozório, R.O., 2016. Role of dietary seaweed supplementation on growth performance, digestive capacity and immune and stress responsiveness in European seabass (*Dicentrarchus labrax*). *Aquac. Rep.* 3, 189-197.
- Pereira, G., Pereira, S.A., Soares, A., Mourão, J.L.P., Merrifield, D., 2019. Autochthonous probiotic bacteria modulate intestinal microbiota of Pirarucu, *Arapaima gigas*. *J. World Aquacult. Soc.* 50, 1152-1167.
- Poupon, R., 2015. Liver alkaline phosphatase: a missing link between cholestasis and biliary inflammation. *Hepatology*, 61(6), 2080-2090.
- Reda RM, El-Hady MA, Selim KM, El-Sayed HM (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.
<https://doi.org/10.1016/j.fsi.2018.06.031>

Reed LV, Muench H (1938) A simple method of estimating fifty percent end points. *Am J Hyg* 27:493–497

Sangma, T., & Kamilya, D. (2015). Dietary *Bacillus subtilis* FPTB13 and chitin, single or combined, modulate systemic and cutaneous mucosal immunity and resistance of catla, *Catla catla* (Hamilton) against edwardsiellosis. *Comparative immunology, microbiology and infectious diseases*, 43, 8-15.

Sareyyupoglu, B., Cantekin, Z., 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. *Vet. Fak Derg.* 57 (15), 157–160.

Shastry, R. P., Dolan, S. K., Abdelhamid, Y., Vittal, R. R., & 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.

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., & 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., 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*) fingerlings. *Aquaculture* 426, 14–20.

Thy, H.T.T., Tri, N.N., Quy, O.M., Fotedar, R., Kannika, K., Unajak, S., 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 catfish (*Pangasianodon hypophthalmus*). *Fish Shellfish Immunol.* 60, 391–399.

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. *J. Fish Dis*, 42(4), 477-487.

Van der Veen, B.S., de Winther, M.P., Heeringa, P., 2009. Myeloperoxidase: molecular mechanisms of action and their relevance to human health and disease. *Antioxid. Redox Signal*. 11 (11), 2899–2937.

Van Kessel, M. A., Mesman, R. J., Arshad, A., Metz, J. R., Spanings, F. T., van Dalen, S. C., ... & Op den Camp, H. J. (2016). Branchial nitrogen cycle symbionts can remove ammonia in fish gills. *Environmental microbiology reports*, 8(5), 590-594.

Wang, S.J., Xu, H.Z. and Xiao, H.L., 2008. Effect of high-frequency electroacupuncture on lipid metabolism in obesity rats. *Zhen ci yan jiu*, 33(3), 154-158.

Wang, Y., Wu, Y., Wang, Y., Xu, H., Mei, X., Yu, D., Wang, Y., Li, W., 2017b. Antioxidant properties of probiotic bacteria. *Nutrients* 9 (5), 521.

Winterbourn, C.C., Kettle, A.J., 2013. Redox reactions and microbial killing in the neutrophil phagosome. *Antioxid. Redox Signal*. 18 (6), 642–660.

Xavier, K. B., & Bassler, B. L. (2005). Interference with AI-2-mediated bacterial cell–cell communication. *Nature*, 437(7059), 750-753.

Yang, G., Shen, K., Yu, R., Wu, Q., Yan, Q., Chen, W., Ding, L., Kumar, V., Wen, C., Peng, M., 2020. Probiotic (*Bacillus cereus*) enhanced growth of Pengze crucian carp by modulating the antioxidant defense response and exerting beneficial impacts on inflammatory response via Nrf2 activation. *Aquaculture* 529, 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 shellfish immun*, 79, 303-312.

Zang, L., Ma, Y., Huang, W., Ling, Y., Sun, L., Wang, X., Zeng, A., Dahlgren, R.A., Wang, C. and Wang, H., 2019. Dietary *Lactobacillus plantarum* ST-III alleviates the toxic effects of triclosan on zebrafish (*Danio rerio*) via gut microbiota modulation. *Fish and shellfish immunology*, 84, pp. 1157-1169. <https://doi.org/10.1016/j.fsi.2018.11.007>

Zhai, Q., Yu, L., Li, T., Zhu, J., Zhang, C., Zhao, J., ... & Chen, W. (2017). Effect of dietary probiotic supplementation on intestinal microbiota and physiological conditions of Nile tilapia (*Oreochromis niloticus*) under waterborne cadmium exposure. *Antonie Van Leeuwenhoek*, 110, 501-513.

Zhang, C.N., Zhang, J.L., Guan, W.C., Zhang, X.F., Guan, S.H., Zeng, Q.H., Cui, W., 2017. Effects of *Lactobacillus delbrueckii* on immune response, disease resistance against *Aeromonas hydrophila*, antioxidant capability and growth performance of *Cyprinus carpio* Huanghe var. *Fish Shellfish Immunol.* 68, 84–91.