Research Article

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

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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 1×10^9 cfu g^{-1} of *C. freundii* (QQ1, G1), *B. foraminis* (QQ2,r G2), *L. plantarum* (without characteristics QQ, WQQ, G3), QQ1 + QQ2 (G4), QQ1 + QQ2+WQQ (combine, G5), and a control 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

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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 benefcial 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 word 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

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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 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

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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 whithout 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

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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)	1×109	1×109	1×109	1×109	1×109	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 (30009g, 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 Fur 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).

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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) × 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 ± 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

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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

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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}

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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

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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 *Enteroccus 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

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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

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probiotic groups and lower levels of ALP may be du 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.

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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.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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