Aquaculture xxx (xxxx) xxxx

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

The effects of host-associated *Enterococcus faecium* CGMCC1.2136 on serum immune parameters, digestive enzymes activity and growth performance of the Caspian roach (*Rutilus rutilus caspicus*) fingerlings

Reza Tarkhani[a], Ahmad Imani[a,*], Seyed Hossein Hoseinifar[b], Kourosh Sarvi Moghanlou[a], Ramin Manaffar[a]

[a] Dept. of Fisheries, Faculty of Agriculture and Natural Resources, Urmia University, I.R. of Iran
[b] Department of Fisheries, Faculty of Fisheries and Environmental Sciences, Gorgan University of Agricultural Sciences and Natural Resources, Iran

ARTICLE INFO

Keywords:
Innate immunity
The Caspian roach
Host-associated probiotic
Enterococcus faecium

ABSTRACT

The effect of different dietary supplementation levels of host-associated *Enterococcus faecium* strain CGMCC1.2136 on the Caspian roach fingerlings was investigated. Three hundred and sixty fish with an average body weight of 12 g were randomly distributed into four treatments receiving different dietary inclusion levels (0 [control], 10^6, 10^7 and 10^8 CFU g^{-1} feed) of *E. faecium* CGMCC1.2136. After an eight-week feeding trial, growth performance, proximate body composition, serum innate immunity and digestive enzymes activity were considered. Results showed that use of different levels of the bacterium significantly improved growth indices ($P < .05$). Those fish fed diet containing 10^8 *E. faecium* CFU g^{-1} feed showed the highest final weight, weight gain and SGR along with the lowest FCR in comparison to control group ($P < .05$). Body protein content of the experimental groups received 10^7 and 10^8 *E. faecium* CFU g^{-1} feed significantly increased ($P < .05$). Dietary inclusion of 10^6 and 10^7 *E. faecium* CFU g^{-1} significantly increased serum total immunoglobulin content of the fingerlings ($P < .05$). Feeding with diet containing 10^8 *E. faecium* CFU g^{-1} resulted in significantly increased serum alkaline phosphatase activity ($P < .05$). Intestinal digestive enzymes activity of those fish received diet included with 10^7 and 10^8 *E. faecium* CFU g^{-1} feed significantly increased ($P < .05$). Moreover, the highest activity of lipase and protease were noticed in those fish received 10^8 *E. faecium* CFU g^{-1} feed ($P < .05$). In conclusion, our results revealed beneficial effects of the host-associated bacterium isolated from adult Caspian roach intestine on growth performance, body composition, non-specific immunity and digestive enzymes activity of the fingerlings.

1. Introduction

With an annual growth rate of 6%, the aquaculture industry had the highest growth rates among the various worldwide animal protein-producing sectors. The industry supplies about 50% of the world's fish consumption, which seems to increase 62% by 2030 (FAO, 2016). Such a great contribution would be achieved via higher stocking density or diversification of aquaculture species. Therefore, the incidence and prevalence of disease and nutritional deficiency constraints are among the major challenges facing the aquaculture industry (Bondad-Reantaso et al., 2005; Doan et al., 2017). The use of feed additives and chemical agents, in particular antibiotics, is a common practice in diseases prevention and control (Dadar et al., 2017). However, due to adverse effects of antibiotics on the environment and human health including the emergence of antibiotic resistant bacteria, accumulation of antibiotic residuals in aquaculture products, and suppression of immune system (Dawood and Koshio, 2016), the use of such compounds has legally been restricted (Dawood and Koshio, 2016; Hoseinifar et al., 2018). It has been predicted that extra 10 million people will be killed each year due to drug-resistant infectious disease by 2050; overtaking cancer. Accordingly, antibiotic resistance will cost the world $ 100 trillion, therefore 2% to 3.5% reduction in Gross Domestic Product (GDP) would be inevitable (O'neill, 2014). The main culprit is irresponsible use of antibiotics in livestock farms. For instance, depending on country and region, farm application of antibiotics accounts for something around 40–70% of all antibiotics used (FAO, 2016). In this context, extensive worldwide research and worthwhile investment have been made on discovering and/or introducing healthy feed additives by private or

* Corresponding author at: Nazlou Campus, 11th km of Serow Road, Urmia, Iran.
E-mail address: a.imani@urmia.ac.ir (A. Imani).

https://doi.org/10.1016/j.aquaculture.2019.734741
Received 7 August 2019; Received in revised form 12 November 2019; Accepted 13 November 2019
Available online 15 November 2019
0044-8486/

governmental sectors (Song et al., 2014; Ringø et al., 2014; Ringø et al., 2016; Guardiola et al., 2016; Carbone and Faggio, 2016; Ringø et al., 2018). Probiotics along with prebiotics and phytogenic compounds are on the top of the list of such feed additives (Van Hai, 2015; Awad and Awaad, 2017; Ringø et al., 2018).

Probiotics via improving digestive enzymes synthesis/secretion, deliberation of inhibitory compounds, competitive exclusion for nutrients or receptor sites on the gut wall along with stimulating host immune system improve fish performance and health (Akhter et al., 2015; Dawood and Koshio, 2016; Wang et al., 2016; Doan et al., 2017; Ringø et al., 2018). Lactic acid bacteria including *Enterococci* are among the well-known and most studied potential probiotics (Ringø et al., 2018). As a commensal human and animal intestinal bacterial flora, *Enterococcus* have been used as potential probiotics in various fish species due to good tolerance to acidic pH of stomach, bile salts, adherence to intestinal epithelial surface, immune modulation and antagonistic activity toward enteropathogens (Rodrigues-Estrada et al., 2009, 2013; Carnevali et al., 2014). For instance, *Enterococcus faecium SF68* proved to be more efficient in decreasing the European eel (*Anguilla anguilla* L) mortality due to edwardsielosis (Chang and Liu, 2002). In a study on the effect of various probiotic bacteria on growth performance and feed utilization of rainbow trout after antibiotic treatment, it has been shown that dietary *E. faecium* supplementation has improved SGR and FCR of the fish (Merrifield et al., 2010a). Of note, they are not predominant in the digestive tract of fish and should be supplied via feed or rearing water (Li et al., 2018).

Unfortunately, most of these probiotics are originated from milk, cheese and other terrestrial sources (Van Doan et al., 2018). Despite of constant and favorable outcomes in higher vertebrates, their role as modifiers of the fish gut microbiota is quite variable mainly due to considerable taxonomical and ecological differences of fish species. Therefore, the use of host-originated microorganisms as probiotics has been gained particular attention since they are more likely to adhere to target animal intestine and appear more effective in terms of stability of beneficial effects in quite variable fish species and culture environment (Lazado et al., 2015; Montalban-Arques et al., 2015; Mukherjee et al., 2019). Furthermore, it has recently been postulated that foreign probiotic bacteria might become pathogenic and produce virulence factors under different intestinal environment of new host via genome reshuffling to adapt to the changing environment (Kothari et al., 2019).

The Caspian roach is one of the most important and most valuable species of the Caspian Sea (Soleimani et al., 2012). However, the species is threatened due to overfishing and subjected to artificial reproduction and re-stocking measures (Rufchaei et al., 2017). To improve the efficiency of re-stocking effort, it is important to increase the fish survival rate and therefore increase its recruitment when introducing to the new environment, i.e. nature. In this sense, probiotic organism could be helpful to enhance the fingerlings immunity and performance facing a new challenging environment (Montalban-Arques et al., 2015). The present study aimed at investigating the effect of dietary inclusion of the isolated lactic acid bacterium from wild adult Caspian roach intestine on growth performance, proximate body composition, innate immune parameters and digestive enzymes activity of the fingerlings.

2. Materials and methods

2.1. Fish husbandry and experimental diets

After one week of quarantine, 360 Caspian roach fingerlings with an average weight of 12.0 g ± 1.0 (Mean ± SD) were randomly allotted to 12 rectangular reservoirs of 120-l containing 100 l aerated artesian water (with a minimum flow rate of 1 L min^{-1}) in a flow-through system without any water mixing among various experimental units to avoid any bacterial transfer among experimental units. Fish stocked at a density of 30 individuals per reservoir with a constant gentle built-in aeration device. They received a commercial diet devoid of any bacterial supplements (Table 1) during a two-week acclimation period at 0.5% of their initial body weight (as a maintenance ratio) to assure that fish were well-acclimated to the experimental units and also evacuate gastrointestinal tract of fish from previous diet and its associated microflora.

Table 1
Formulation and proximate composition of commercial (or basal) diet used in this research.

Ingredient	%	Ingredient	%
Soybean meal	46.0	Mineral premix	1.0
Wheat flour	34.0	Stay C (35%)	0.2
Fish meal	6.0	Choline chloride (60%)	0.2
Fish oil	2.5	Methionine	0.2
Soybean oil	3.0	BHT	0.1
Calcium diphosphate	2.5	Bentonite	2.0
Vitamin premix	1.0	Cellulose	1.3
Proximate composition			
Moisture	8.0	Ash	12.0
Crud protein	40.0	Nitrogen-free extract	24.0
Crud lipid	11.0	Crude energy (kcal/kg)	4487
Fiber	5.0		

Composition of vitamin premix (IU, g or mg/kg): A (3,600,000 IU), D_3 (8,000,000 IU), E (14.4 g), K_3 (800 mg), B_1 (7 g), B_2 (2.64 g), Niacin (11.8 g), Calcium pantothenate (3.92 g), B_6 (1.17 g), B_9 (0.4 g), Biotin (40 mg), Choline chloride (100,000 mg). Aras Bazar Pharmaceutical Company, Iran.
Composition of mineral premix (g/kg): Mn (39.68 g), Fe (20 g), Zn (33.88 g), Co (4 g), I (0.39 g), Se (0.08 g). Aras Bazar Pharmaceutical Company, Iran.
Nitrogen-free extract = 100 − (protein + lipid + ash +fiber + moisture).

A host-associated probiotic used in the present study has already been isolated from adult Caspian roach intestinal microbiota and characterized as a putative probiotic (Kos et al., 2003; Balcázar et al., 2008; Sriphannam et al., 2012; Mukherjee et al., 2019). After morphologic and biochemical characterization, the bacterium was finally indentified by PCR-sequencing of 16sRNA gene as *Enterococcus faecium* strain CGMCC1.2136 (Acc. No. AJKH01000109) (Saitou and Nei, 1987; Kumar et al., 2016).

A completely randomized trial comprised of four experimental groups including various dietary supplementation of the isolate, namely, 0 (as a control group), 10^6, 10^7 and 10^8 CFU g^{-1} feed, nominated as C, P106, P107 and P108, respectively, were designated and carried out for eight weeks. The experimental groups were in triplicate. The overnight culture of the isolate was harvested by centrifugation at 6000 ×*g* for 20 min at 4 °C. To remove the culture media, the procedure repeated three times using sterile normal saline solution and the resultant bacteria was suspended in the same solution before spraying onto the basal diet (Van Doan et al., 2018). A desired weight of the basal diet was spread on a tray as a monolayer and the isolate suspension was sprayed onto pellets using fine mist spray to achieve the desired bacterial load on each diet. Also, the same volume of the sterile saline solution was sprayed onto the control diet. Finally, all diets were coated using 1% bovine gelatine solution. Letting the pellets get dried in room temperature, they were sealed in bags, stored at 4 °C and used within one week (Irianto and Austin, 2002). The probiotic supplementation levels were based on previous studies on *E. faecium* (Swain et al., 2009; Sun et al., 2011; Allameh et al., 2015). Fish were fed two times a day at 3% of their respective body weight. Every fortnight, total biomass of each reservoir was determined after anesthetizing 24 h-food deprived fish with clove oil solution 0.5 g l^{-1} (Safari et al., 2016) to adjust feeding rate. The photoperiod of 15 h light to 9 h dark was employed and all water quality criteria including pH = 7.6–7.9, DO = 8.5 ± 0.12 ppm and temperature = 17.3 ± 0.2 °C were monitored every day and were within the optimum prerequisites for the fish.

2.2. Growth performance and proximate body composition

Fish were weighed to nearest 0.1 g after clove oil induced anesthesia. Simultaneously, whole fish samples were also taken (five fish from each treatment) and stored at −20 °C for later proximate body composition analyses according to Peterson et al. (1999). Growth performance indices were calculated as follow (Farhangi and Carter, 2001):

Weight gain (WG,%) = $(W_2 - W_1)/W_1 \times 100$

Specific growth rate (SGR, %day^{-1}) = $(\ln W_2 - \ln W_1) / t \times 100$, where W_1, W_2 and t were initial and final mean body weight and length of experimental period (day), respectively.

Feed conversion ratio (FCR) = g feed intake/g live weight gain

Protein efficiency ratio (PER) = g weigh gain/g protein intake

Survival rate (%) = $N_f/N_i \times 100$, where N_i and N_f were initial and final fish counts of each reservoir, respectively.

2.3. Innate immunity

At the end of experiment, three fish from each reservoir were randomly taken and anesthetized by clove oil. Blood was collected from the caudal vein using 5 ml non-heparinized syringes. Sera were separated by centrifugation (Heraeus Labofuge 400) at 1600 g for 10 min and stored at −20 °C for further analyses.

Serum lysozyme activity was determined according to the method described by Ellis (1990) with a slight modification using lyophilized *Micrococcus lysodeikticus* (4698 ATCC No.) suspension. Serum total protein content was determined according to Bradford (1976) using a commercial kit (Pars-Azmun Co., Tehran, Iran). Total immunoglobulin content of serum samples was also determined according to Siwicki and Anderson (1993). Alternative complement activity (ACH50) was assayed based on hemolysis of rabbit RBCs (Sunyer and Tort, 1995). Finally, Alkaline phosphatase activity of serum samples was calorimetrically determined using 4-nitrophenol phosphate as a substrate and diethanolamine (DEA) as a buffer according to DGKC (Deutsche Gesellschaft für Klinische Chemie) (Pars-Azmun Co., Tehran, Iran).

2.4. Digestive enzymes

2.4.1. Sampling and crude enzyme extract preparation

Specific activities of selected digestive enzymes including alkaline protease, lipase and amylase were measured at the end of the experiment. Intestine samples were randomly taken after 24 h of feed deprivation. Fish were anesthetized by clove oil and euthanized by severing the spinal cord. Intestine was washed by cold normal saline solution and stored at −80 °C until crude enzyme extract preparation. All sampling procedures were carried out on ice-cold trays (Lemieux et al., 1999).

The tissues were homogenized in 1:3 (*w/v*) cold 50 mM tris-HCl buffer, pH = 7.5, using Polytron PT 1300 D homogenizer with a 7 mm generator at a setting of 10 for 3 × 30s. After centrifuging homogenates at 10000 g for 20 min, 4 °C, the supernatants were collected and stored at −80 °C until enzymes activity assays (Chang and Liu, 2002).

2.4.2. Enzyme assays

Alkaline protease activity was determined according to Garcia-Carreno and Haard (1993) using 2% Azocasein solution in Tris-HCl, pH = 7.5 as the substrate. The reaction was terminated using 0.5 ml of 20% Trichloroacetic acid (TCA) after 10 min of incubation at 25 °C and the optical density of the supernatant was determined at 440 nm. Finally, the enzyme activity was reported as U mg protein^{-1} min^{-1}.

Hydrolysis of ρ-nitrophenyl myristate, as the substrate, was recorded at 405 nm to determine the specific activity of lipase. In Brief, each assay contained 0.53 mM *p*-nitrophenyl myristate, 0.25 mM 2-methoxyethanol, 5 mM sodium cholate and 0.25 M Tris–HCl (pH = 9.0) at 25 °C. The reaction was halted after 15 min by adding 0.7 ml of acetone/n-heptane (5:2, *v*/v). The optical density of the aqueous solution was recorded after centrifuging the mixture at 6080 g for 2 min. Unit enzyme activity was expressed as 1 μmol of ρ-nitrophenol released mg protein^{-1} min^{-1} (Iijima et al., 1998).

Using starch as a substrate, α-amylase activity was determined based on the method developed by Bernfeld (1955). In Brief, 1% *w/v* starch solution in 0.02 M sodium phosphate buffer containing 0.006 M NaCl (pH = 6.9) was incubated with crude enzyme extract for 4 min at 25 °C. Finally, 0.5 ml of dinitrosalicylic acid (DNS) solution (1% *w/v*) was added to the mixture. The final reaction cocktail was boiled for 5 min and cooled down at room temperature. Optical density was measured after addition of 5 ml distilled water to the cocktail and specific activity of α-amylase was expressed as μmol of maltose released mg protein^{-1} min^{-1} at 25 °C. Total soluble protein content of the crude extracts was determined according to Bradford (1976) using bovine serum albumin as standard.

2.5. Statistical procedures

The homoscedasticity of variances was evaluated using Levene's test. Standard normality test of Kolmogorov-Smirnov was applied to determine normality of dataset. One-way ANOVA was used to elucidate significant differences among different experimental groups. Tukey's HSD test was used for multiple comparisons. All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 20.0 (IBM Corp, Armonk, NY, USA) at the significance level of < 0.05. Results were reported as Mean ± SD.

3. Results

3.1. Growth performance and proximate body composition

The effect of dietary *E. faecium* CGMCC1.2136 supplementation on growth performance of Caspian roach fingerlings was presented in Table 2. Feeding on probiotic supplemented diets significantly improved weight gain, SGR, FCR and PER of the Caspian roach fingerlings ($P < .05$, Table 2). Despite the lack of significant differences among the groups received various dietary supplementation of the isolate,

Table 2
The effects of different levels of dietary *E. faecium* CGMCC1.2136 on growth performance and survival rate of Caspian roach fingerling.

	C	P106	P107	P108
Initial weight (g)	12.779 ± 0.06^{a} *	12.762 ± 0.02 ^{a}	12.805 ± 0.06 ^{a}	12.808 ± 0.03 ^{a}
Final weight (g)	24.577 ± 0.42 ^{b}	27.266 ± 0.13 ^{a}	27.377 ± 0.91 ^{a}	28.111 ± 0.16 ^{a}
WG (%)	92.333 ± 3.92 ^{b}	113.655 ± 1.17 ^{a}	113.774 ± 6.15 ^{a}	119.472 ± 1.77 ^{a}
SGR (% day^{-1})	1.089 ± 0.03 ^{b}	1.265 ± 0.009 ^{a}	1.265 ± 0.04 ^{a}	1.31 ± 0.01 ^{a}
FCR	2.488 ± 0.09 ^{a}	2.022 ± 0.01 ^{b}	2.017 ± 0.12 ^{b}	1.917 ± 0.02 ^{b}
PER	1.006 ± 0.04 ^{b}	1.236 ± 0.01 ^{a}	1.242 ± 0.07 ^{a}	1.304 ± 0.02 ^{a}
Survival (%)	100	100	100	100

* Values are mean ± S.D. and different letters in each row denote significant differences ($P < .05$).

Table 3
The effects of different levels of dietary *E. faecium* CGMCC1.2136 on proximate body composition of Caspian roach fingerlings.

	C	P106	P107	P108
Protein (g/100 g)	14.227 ± 0.07 ^{b}*	13.777 ± 0.23 ^{ab}	15.013 ± 0.2 ^{a}	15.65 ± 0.16 ^{a}
Lipid (g/100 g)	7.433 ± 0.1 ^{a}	7.457 ± 0.33 ^{a}	7.137 ± 0.2 ^{a}	7.467 ± 0.25 ^{a}
Moisture (g/100 g)	75.113 ± 0.2 ^{ab}	75.587 ± 0.4 ^{ab}	74.46 ± 0.3 ^{b}	72.773 ± 0.3 ^{c}
Ash (g/100 g)	3.163 ± 0.08 ^{b}	3.113 ± 0.12 ^{b}	3.32 ± 0.1 ^{ab}	3.987 ± 0.31 ^{a}

* Values are mean ± S.D. and different letters in each row denote significant differences ($P < .05$).

including 10^8 *E. faecium* CFU g^{-1} feed (i.e., treatment P108) resulted in better growth performance indices.

The whole proximate body composition of various experimental groups was presented in Table 3. The results showed no significant difference in carcass lipid content of different treatments ($P > .05$). The highest body protein contents were observed in treatments P107 and P108, which were significantly higher than control group and treatment P106 ($P < .05$). The lowest moisture content was observed in treatment P108 ($P < .05$). In addition, the highest percentage of carcass ash content was observed in treatment P108 (P < .05).

3.2. Innate immunity

The effects of supplementing different levels of *E. faecium* CGMCC1.2136 on the serum total protein was shown in Fig. 1A. Those fish received probiotic supplemented diets (regardless of inclusion level) had higher serum protein content in comparison to control group. The highest serum total protein content was belonged to P106 group, however, there were no significant differences among groups received *E. faecium* CGMCC1.2136 ($P > .05$). Serum total immunoglobulin content of experimental groups was depicted in Fig. 1B. Similarly, serum total immunoglobulin content of groups P106 and P107 were significantly higher than that of control group ($P < .05$); the highest total immunoglobulin content.

The effect of dietary supplementation with the isolate on the serum lysozyme activity was shown in Fig. 1C. The results revealed significant increase of lysozyme activity in treatments P107 and P108 (P < .05). No significant difference was noticed between treatment P106 and control group ($P > .05$).

Evaluation of the serum ACH50 showed notable difference between fish fed the isolate supplemented diets and the group fed control diet (P < .05). The highest activity was noticed in treatment P107 (Fig. 2A, P < .05).

Fig. 2B represented the effects of different dietary supplementation levels of *E. faecium* CGMCC1.2136 on the serum alkaline phosphatase activity of the Caspian roach fingerlings. Regardless of dietary supplementation level, those fish fed probiotic containing diet showed significantly higher enzyme activity ($P < .05$).

3.3. Digestive enzymes

The effects of feeding on diets containing different levels of *E. faecium* CGMCC1.2136 were brought in Table 4. The highest activity of all

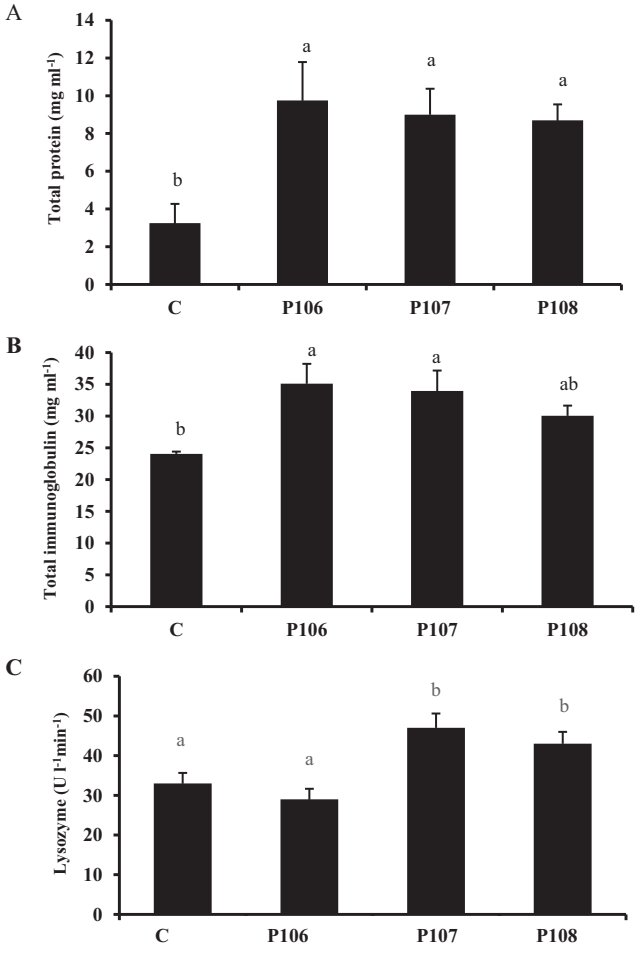

Fig. 1. The effects of different levels of dietary *E. faecium* CGMCC1.2136 on serum (A) total protein content, (B) total immunoglobulin content and (C) lysozyme activity of Caspian roach fingerlings. Values are mean ± SD. Different letters indicate statistically significant differences at $P < .05$.

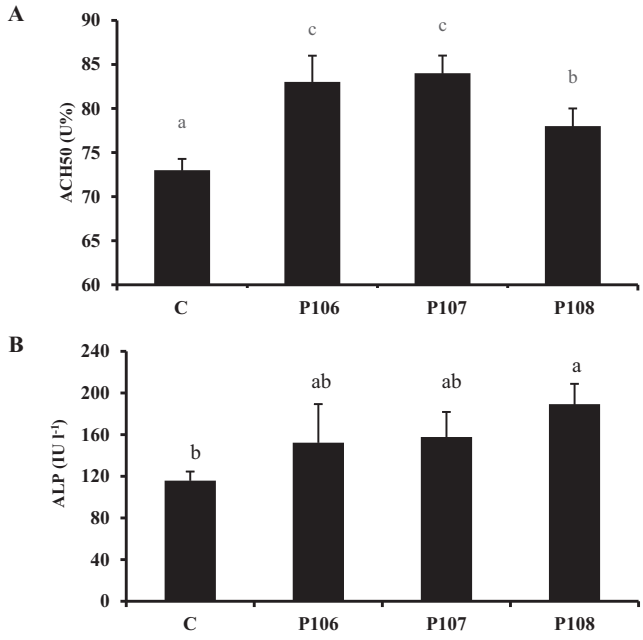

Fig. 2. The effects of different levels of dietary *E. faecium* CGMCC1.2136 on serum (A) ACH50 and (B) alkaline phosphatase activity of Caspian roach fingerlings. Values are mean ± SD. Different letters indicate statistically significant differences at P < .05.

Table 4
The effects of different levels of dietary *E. faecium* CGMCC1.2136 supplementation on digestive enzymes activity of Caspian roach fingerlings.

	C	P106	P107	P108
Amylase (μmole maltose/mg protein/min)	27.99 ± 0.81^{b}*	27.87 ± 2.70^{b}	42.80 ± 2.32^{a}	48.06 ± 2.45^{a}
Lipase (μmole ρ-nitrophenol/mg protein/min)	2.21 ± 0.04^{b}	1.43 ± 0.10^{c}	2.77 ± 0.14^{ab}	3.12 ± 0.15^{a}
Protease (U/mg protein/min)	9.64 ± 0.5^{bc}	7.8 ± 0.24^{c}	11.33 ± 0.34^{ab}	12.63 ± 0.44^{a}

* Values are mean ± S.D. and different letters in each row denote significant differences ($P < .05$).

three digestive enzymes (amylase, lipase and alkaline protease) were observed in group P108, as it significantly differed from control group ($P < .05$). Although, no significant difference was noticed between treatments P107 and P108 regarding amylase activity (Table 4, $P > .05$).

4. Discussion

Identification and introduction of new beneficial bacterial microorganisms with aquatic animal origins would be very useful for animal health management and performance in commercial aquaculture. Besides increasing growth performance and feed efficiency, probiotics are capable of strengthening host immune system and considered promising alternatives for antibiotics (Chauhan and Singh, 2019).

The growth and nutritional indices of the Caspian roach fingerlings fed diets containing host-associated *E. faecium* CGMCC1.2136 significantly improved. Such improvements in growth performance have been attributed to increased absorption and improved nutritional efficiency of the animal (Giri et al., 2013; Ramesh et al., 2017). Allameh et al. (2015) reported that dietary supplementation of *E. faecium* increased growth indices of Japanese carp (*Puntius gonionotus*), which was attributed to increased activity of digestive enzymes and improved nutrient digestion and absorption. The finding was in line with the present study as dietary probiotic administration significantly increased digestive enzymes activity. Besides, Merrifield et al. (2010b) reported that feeding trout with *E. faecium* decreased FCR and improved PER as we noticed the same in the case of the Caspian roach fingerlings in the current study. Furthermore, supplementation of tilapia (Wang et al., 2008), catfish (*Silurus glanis*) (Bogut et al., 2000) and *Epinephelus coioides* (Sun et al., 2011) diets with *E. faecium* significantly increased growth performance of the species. Similar to our findings, Mukherjee et al. (2019) feeding *Labeo rohita* fingerlings on diet supplemented with single or conjoint putative autochthonous probiotic bacillus species reported that those fish received combination of *B. methylotrophicus* and *B. licheniformis* showed improvement SGR, FCR, WG and PER. Moreover, dietary supplementation of different levels of *E. faecium* CGMCC1.2136 increased protein content of the carcass or in another word increased body protein retention. However, there were no significant differences in body lipid content of various experimental groups. Similarly, higher carcass protein content were reported in rainbow trout (Heidarieh et al., 2013) and Beluga (*Huso huso*) (Hasanpour Fattahi et al., 2014) fed with probiotic as a feed supplement. Some researchers believe that changes in proximate body composition could be attributed to changes in nutrient absorption and/or body retention and also to changes in animal growth rates (Abdel-Tawwab et al., 2008; Sheikhzadeh et al., 2012). In a good accordance, similar results have been reported following supplementation of Catla (*Catla catla*) and Japanese flounder diets with probiotics (*Bacillus circulans*) (Bandyopadhyay and Das Mohapatra, 2009; Ye et al., 2011).

E. faecium is one of the natural bacteria populations of intestinal microbiota of human and other animals such as fish (Chang and Liu, 2002). Gatesoupe (1999) reported that *E. faecium* can be used as an appropriate probiotic to control the harmful effects of *Edwardsiella tarda*, which is one of the most important marine fish pathogens. The present study revealed that dietary administration of the isolate significantly increased serum lysozyme and complement activity. This increment shows the immunomodulatory effects of the bacterium. In accordance with present findings, Kim et al. (2012) pointed out that *E. faecium* increased the immune parameters including lysozyme, complement and protease activities in Japanese flounder (*Paralichthys olivaceus*). Immunoglobulins provide prompt and broad-range protection against pathogens, which make them a vital part of the non-specific immune system (Magnadottir, 2010). Our results showed that serum immunoglobulin levels of fish fed with 10^6 and 10^7 CFU *E. faecium*.g^{-1} feed compared to the control group, which was in line with findings reported by Sun et al. (2011). The increase of serum immunoglobulin levels has been reported in various studies following the use of immunostimulants (Nayak et al., 2007; Mohapatra et al., 2011; Sun et al., 2011; Hosseini et al., 2016; Liu et al., 2017). In addition, Allameh et al. (2015) and Mukherjee et al. (2019) found that supplementation of Japanese carp diet with *E. faecium* and L. *rohita* with various bacillus species isolated form the fish gut microbiota, respectively, improved immune parameters of the species. Also, Nikoskelainen et al. (2001) demonstrated that the host-associated lactic acid bacteria stimulated immunoglobulin secretion. The increase of total immunoglobulin content of serum possibly indicated modulation of immune parameters in probiotic-fed fish. Various factors including adhesion sites, stressors, nutritional status and the environment play a role in colonizing probiotics in the host gut (Li et al., 2018). Intestine is an active site for immunity due to presence of gut associated lymphoid tissue (Lazado and Caipang, 2014) and probiotics are believed to interact with the lymphoid tissue to stimulate host immune system (Caipang and Lazado, 2015). Serum alkaline phosphatase is known to be an antibacterial agent due to its hydrolytic activity. This enzyme also plays an important role in wound healing and control of parasitic infections (Ross et al., 2000; Subramanian et al., 2007). In this study, serum alkaline phosphatase activity of probiotic-fed fish was higher than that of the control group and it was significantly higher in those fish received 10^8 CFU g^{-1} feed supplemented diet. Likewise, the use of probiotic *Lactobacillus acidophilus* in tiger barb (*Puntigrus tetrazona*) increased the activity of alkaline phosphatase in skin mucus (Roosta and Hoseinifar, 2016). The same results were also reported by Sheikhzadeh et al. (2012) following feeding rainbow trout (*Oncorhynchus mykiss*) with probiotic *Saccharomyces cerevisiae* and feeding probiotic *Lactobacillus acidophilus* to sword tail (*Xiphophorus hellerii*) (Hoseinifar et al., 2015). Improvement of the immune system caused by probiotics, as observed in the present study, might be attributable to modulation of intestinal microbiota and increase of lactic acid bacteria population with immune-stimulating properties (Song et al., 2014; Nawaz et al., 2018).

Activity of digestive enzymes is an indicator of digestive function and nutritional status of fish. Their activity could be affected by any dietary changes (Mohapatra et al., 2011). Our results showed that the supplementation of *E. faecium* CGMCC1.2136 notably increased the activity of intestinal lipase, alkaline protease and amylase. Various studies have shown that some probiotics secreted extracellular enzymes such as amylase, lipase and protease, and have the ability to be supplemented to fish diets in order to increase the absorption of nutrients and improve body nutrients mainly protein accretion (Dawood and Koshio, 2016; Hoseinifar et al., 2017; Mukherjee et al., 2019). Similarly, inclusion of photosynthetic bacteria along with *Bacillus* sp. and *Lactobacillus* spp. in common carp (*Cyprinus carpio*) and sea bream larvae (*Sparus aurata* L.) diets, respectively, improved digestive enzyme

activity of the species (Yanbo and Zirong, 2006; Suzer et al., 2008). It has been proposed that probiotic bacteria increase the digestion and absorption of nutrients via stimulating the secretion of host digestive enzymes or by their exogenous enzymes (Ziaei-Nejad et al., 2006). The use of *Pediococcus acidilactici* in Pollack (*Pollachinus pollachinus*) resulted in increased digestive enzymes activity and better growth and food utilization (Gatesoupe, 2002).

In conclusion, the present study showed that *E. faecium* CGMCC1.2136 had beneficial effects on growth performance, body protein content, innate immunity and digestive enzymes activity of the Caspian roach fingerlings. The bacterium could be used as a promising feed supplement for enhancing immune competence and growth performance of the fingerlings in the re-stocking programme before releasing to the stressful natural environment.

Declaration of Competing Interest

We declare that all authors declare no conflicts of interest.

Acknowledgements

Authors express their sincere gratitude to Research Council of Urmia University for financial support of the research.

References

Abdel-Tawwab, M., Abdel-Rahman, A.M., Ismael, N.E.M., 2008. Evaluation of commercial live bakers' yeast, *Saccharomyces cerevisiae* as a growth and immunity promoter for fry Nile tilapia, *Oreochromis niloticus* (L.) challenged *in situ* with *Aeromonas hydrophila*. Aquaculture. 280, 185–189.

Akhter, N., Wu, B., Memon, A.M., Mohsin, M., 2015. Probiotics and prebiotics associated with aquaculture: a review. Fish. Shellfish Immunol. 45, 733–741.

Allameh, S., Ringø, E., Yusoff, F., Daud, H., Ideris, A., 2015. Dietary supplement of *Enterococcus faecalis* on digestive enzyme activities, short-chain fatty acid production, immune system response and disease resistance of Javanese carp (*Puntius gonionotus*, Bleeker 1850). Aquac. Nutr. 29, 12–21.

Awad, E., Awaad, A., 2017. Role of medicinal plants on growth performance and immune status in fish. Fish. Shellfish. Immunol. 67, 40–54.

Balcázar, J.L., Vendrell, D., de Blas, I., Ruiz-Zarzuela, I., Muzquiz, J.L., Girones, O., 2008. Characterization of probiotic properties of lactic acid bacteria isolated from intestinal microbiota of fish. Aquaculture. 278, 188–191.

Bandyopadhyay, P., Das Mohapatra, P.K., 2009. Effect of a probiotic bacterium *Bacillus circulans* PB7 in the formulated diets: on growth, nutritional quality and immunity of *Catla catla* (ham.). Fish Physiol. Biochem. 35, 467–478.

Bernfeld, P., 1955. Amylase. In: Colowick, S.P., Kaplan, N.O. (Eds.), Methods in Enzymology. Academic Press, New York, pp. 149–158.

Bogut, I., Milakovic, Z., Brkic, S., Novoselic, D., Bukvic, Z., 2000. Effects of *Enterococcus faecium* on the growth rate and content of intestinal microflora in sheat fish (*Silurus glanis*). Vet. Med. 45, 107–109.

Bondad-Reantaso, M.G., Subasinghe, R.P., Arthur, J.R., Ogawa, K., Chinabut, S., Adlard, R., 2005. Disease and health management in Asian aquaculture. Vet. Parasitol. 132, 249–272.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the protein-dye binding. Anal. Biochem. 72, 248–254.

Caipang, C.M.A., Lazado, C.C., 2015. Nutritional impacts on fish mucosa: immunostimulants, pre- and probiotics. In: BHB, Peatman (Ed.), Mucosal Health in Aquaculture. Academic Press, San Diego, pp. 211–272.

Carbone, D., Faggio, C., 2016. Importance of prebiotics in aquaculture as immunostimulants. Effects on immune system of *Sparus aurata* and *Dicentrarchus labrax*. Fish. Shellfish Immunol. 54, 172–178.

Carnevali, O., Sun, Y.Z., Merrifield, D.L., Zhou, Z., Picchietti, S., 2014. Probiotic application in temperate and warm water fish species. In: Merrifield, D.L., Ringø, E. (Eds.), Aquaculture Nutrition: Gut Health, Probiotics and Prebiotics. Wiley-Blackwell Publishing, Oxford, UK, pp. 253–289.

Chang, C.I., Liu, W.Y., 2002. An evaluation of two probiotic bacterial strains, *Enterococcus faecium* SF68 and *Bacillus toyoi*, for reducing edwardsiellosis in cultured European eel, *Anguilla anguilla* L. J. Fish Dis. 25, 311–315.

Chauhan, A., Singh, R., 2019. Probiotics in aquaculture: a promising emerging alternative approach. Symbiosis. 77 (2), 99–113.

Dadar, M., Dhama, K., Vakharia, V.N., Hoseinifar, S.H., Karthik, K., Tiwari, R., 2017. Advances in aquaculture vaccines against fish pathogens: global status and current trends. Rev. Fish. Sci. Aquac. 25, 184–217.

Dawood, M.A.O., Koshio, S., 2016. Recent advances in the role of probiotics and prebiotics in carp aquaculture: a review. Aquaculture. 454, 243–251.

Doan, H.V., Hoseinifar, S.H., Tapingkae, W., Chitmanat, C., Mekchay, S., 2017. Effects of *Cordyceps militaris* spent mushroom substrate on mucosal and serum immune parameters, disease resistance and growth performance of Nile tilapia, (*Oreochromis niloticus*). Fish. Shellfish. Immunol. 67, 78–85.

Ellis, T.A., 1990. Lysozyme assays. In: Stolen, J.S., Fletcher, T.C., Anderson, D.P., Roberson, B.S., Van Muiswinkel, W.B. (Eds.), Techniques in Fish Immunology. SOS Publications, Fair Haven, pp. 101–103.

FAO, 2016. The State of World Fisheries and Aquaculture. Food and Agriculture Organization of the United Nations, Rome.

Farhangi, M., Carter, C.G., 2001. Growth, physiological and immunological responses of rainbow trout (*Onchorhynchus mykiss*) to different dietary inclusion levels of dehulled lupin (*Lupins angustifolius*). Aquac. Res. 32, 329–340.

Garcia-Carreno, F.L., Haard, N.F., 1993. Characterization of proteinase classes in *Langostilla pleuroncodesplanipes* and crayfish *Pacifastacus astacus* extracts. Food Biochem. 17, 97–113.

Gatesoupe, F.J., 1999. The use of probiotics in aquaculture. Aquaculture. 180, 147–165.

Gatesoupe, F.J., 2002. Probiotic and formaldehyde treatments of Artemia nauplii as food for larval pollack, *Pollachius pollachius*. Aquaculture. 212, 347–360.

Giri, S.S., 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, 660–666.

Guardiola, F.A., Porcino, C., Cerezuela, R., Cuesta, A., Faggio, C., Esteban, M.A., 2016. Impact of date palm fruits extracts and probiotic enriched diet on antioxidant status, innate immune response and immune-related gene expression of European seabass (*Dicentrarchus labrax*). Fish. Shellfish. Immunol 52, 298–308.

Hasanpour Fattahi, A., Jafaryan, H., Khosravi, A.R., Gholipour Kanani, H., 2014. Effects of *Saccharomyces cerevisiae* and *Aspergillus niger* Isolated from Beluga Adult Digestive Tract on the Feeding Efficiency and Blood Serum Enzymes of Beluga Juvenile Sturgeon (*Huso huso*). J. Fish. Sci. Techol. 3, 1–14.

Heidarieh, M., Mirvaghefi, A.R., Akbari, M., Sheikhzadeh, N., Kamyabi-Moghaddam, Z., Askari, H., 2013. Evaluations of Hilyses™, fermented *Saccharomyces cerevisiae*, on rainbow trout (*Oncorhynchus mykiss*) growth performance, enzymatic activities and gastrointestinal structure. Aquac. Nutr. 19, 343–348.

Hoseinifar, S.H., Roosta, Z., Hajimoradloo, A., Vakili, F., 2015. The effects of *Lactobacillus acidophilus* as feed supplement on skin mucosal immune parameters, intestinal microbiota, stress resistance and growth performance of black swordtail (*Xiphophorus helleri*). Fish. Shellfish. Immunol. 42, 533–538.

Hoseinifar, S.H., Sun, Y.Z., Caipang, C.M., 2017. Short chain fatty acids as feed supplements for sustainable aquaculture: an updated view. Aquac. Res. 48, 1380–1391.

Hoseinifar, S.H., Sun, Y., Wang, A., Zhou, Z., 2018. Probiotics as means of diseases control in aquaculture, a review of current knowledge and future perspectives. Front. Microbiol. 9, 24–29.

Hosseini, M., Kolangi Miandare, H., Hoseinifar, S.H., Yarahmadi, P., 2016. Dietary *Lactobacillus acidophilus* modulated skin mucus protein profile, immune and appetite genes expression in gold fish (*Carassius auratus gibelio*). Fish. Shellfish. Immunol 59, 149–154.

Iijima, N., Tanaka, S., Ota, Y., 1998. Purification and characterization of bile salt-activated lipase from hepatopancrease of red sea bream *Pagarus major*. Fish Physiol. Biochem. 18, 59–69.

Irianto, A., Austin, B., 2002. Use of probiotics to control furunculosis in rainbow trout, *Oncorhynchus mykiss* (Walbaum). Fish. Dis. 25, 333–342.

Kim, Y.R., Kim, E.Y., Choi, S., Hossain, M.T., Oh, R., Heo, W.S., 2012. Effect of a probiotic strain, *Enterococcus faecium*, on the immune responses of olive flounder (*Paralichthys olivaceus*). J. Microbiol. Biotechnol. 22, 526–539.

Kos, B., Suskovic, J., Simpraga, M., Frece, J., Matosic, S., 2003. Adhesion and aggregation ability of probiotic strain *Lactobacillus acidophilus* M92. J. Appl. Microbiol. 94, 981–987.

Kothari, D., Patel, S., Kim, S.K., 2019. Probiotic supplements might not be universally-effective and safe: a review. Biomed. Pharmacother. 111, 537–547.

Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33, 1870–1874.

Lazado, C.C., Caipang, C.M.A., 2014. Mucosal immunity and probiotics in fish. Fish. Shellfish. Immunol. 39, 78–89.

Lazado, C.C., Caipang, C.M.A., Estante, E.G., 2015. Prospects of host-associated microorganisms in fish and penaeids as probiotics with immunomodulatory functions. Fish. Shellfish. Immunol. 45, 2–12.

Lemieux, H., Blier, P., Dutil, J.D., 1999. Do digestive enzymes set a physiological limit on growth rate and food conversion efficiency in the Atlantic cod (*Gadus morhua*). Fish Physiol. Biochem. 20, 293–303.

Li, X., Ringø, E., Hoseinifar, S.H., Lauzon, H., Birkbeck, H., Yang, D., 2018. Adherence and colonisation of microorganisms in the fish gastrointestinal tract. Rev. Aquac. 52, 108–124.

Liu, H., Wang, S., Cai, Y., Guo, X., Cao, Z., Zhang, Y., Liu, S., Yuan, W., Zhu, W., Zheng, Y., Xie, Z., Guo, W., Zhou, Y., 2017. Dietary administration of *Bacillus subtilis* HAINUP40 enhances growth, digestive enzyme activities, innate immune responses and disease resistance of tilapia, *Oreochromis niloticus*. Fish. Shellfish. Immunol 60, 326–333.

Magnadottir, B., 2010. Immunological control of fish diseases. Mar. Biotechnol. 12, 361–379.

Merrifield, D.L., Dimitroglou, A., Bradley, G., Baker, R.T.M., Davies, S.J., 2010a. Probiotic applications for rainbow trout (Oncorhynchus mykiss Walbaum) I. effects on growth performance, feed utilization, intestinal microbiota and related health criteria. Aquac. Nutr. 16, 504–510.

Merrifield, D.L., Dimitroglou, A., Foey, A., Davies, S.J., Baker, R.T.M., Bøgwald, J., Castex, M., Ringø, E., 2010b. The current status and future focus of probiotic and prebiotic applications for salmonids. Aquaculture. 302, 1–18.

Mohapatra, S., Chakraborty, T., Prusty, A., Paniprasad, K., Mohanta, K., 2011. Use of different microbial probiotics in the diet of Rohu (*Labeo rohita*) fingerlings: effects on growth, nutrient digestibility and retention, digestive enzyme activities and intestinal microflora. Aquac. Nutr. 18, 1–11.

Montalban-Arques, A., De Schryver, P., Bossier, P., Gorkiewicz, G., Mulero, V., Gatlin III,

D.M., Galindo-Villegas, J., 2015. Selective manipulation of the gut microbiota improves immune status in vertebrates. Front. Immunol. 6 (512), 1–14.
Mukherjee, A., Chandra, G., 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.
Nawaz, A., Bakhsh javaid, A., Irshad, S., Hoseinifar, S.H., Xiong, H., 2018. The functionality of prebiotics as immunostimulant: evidences from trials on terrestrial and aquatic animals. Fish. Shellfish. Immunol 76, 272–278.
Nayak, S., Swain, P., Mukherjee, S., 2007. Effect of dietary supplementation of probiotic and vitamin C on the immune response of Indian major carp, *Labeo rohita* (ham.). Fish. Shellfish. Immunol. 23, 892–896.
Nikoskelainen, S., Salminen, S., Bylund, G., Ouwehand, A.C., 2001. Characterization of the properties of human-and dairy-derived probiotics for prevention of infectious diseases in fish. Appl. Environ. Microbiol. 67, 2430–2435.
O'neill, J.I.M., 2014. Antimicrobial resistance: tackling a crisis for the health and wealth of nations. Rev. Antimicrobial Res. 20, 1–20.
Peterson, D.S., Harris, D.J., Rayner, J.C., Blakeney, A.B., Choct, M., 1999. Methods for the analysis of premium livestock grains. Agric. Res. 50, 775–787.
Ramesh, D., Souissi, S., Ahamed, T.S., 2017. Effects of the potential probiotics *Bacillus aerophilus* KADR3 in inducing immunity and disease resistance in *Labeo rohita*. Fish. Shellfish. Immunol. 70, 408–415.
Ringø, E., Olsen, R.E., Jensen, I., Romero, J., Lauzon, H.L., 2014. Application of vaccines and dietary supplements in aquaculture: possibilities and challenges. Rev. Fish Biol. Fish. 24, 1005–1032.
Ringø, E., Zhou, Z., Vecino, J.L.G., Wadsworth, S., Romero, J., Krogdahl, Å., 2016. Effect of dietary components on the gut microbiota of aquatic animals. A never-ending story? Aquac. Nutr. 22, 219–282.
Ringø, E., Hoseinifar, S.H., Ghosh, K., Doan, H.V., Beck, B.R., Song, S.K., 2018. Lactic acid Bacteria in finfish-an update. Front. Microbiol. 9, 12–19.
Rodrigues-Estrada, U., Satoh, S., Haga, Y., Fushimi, H., Sweetman, J., 2009. Effects of single and combined supplementation of *Enterococcus faecalis*, mannan oligosaccharide and polyhydroxybutyrate acid on growth performance and immune response of rainbow trout *Oncorhynchus mykiss*. Aquac. Sci. 57, 609–617.
Rodrigues-Estrada, U., Satoh, S., Haga, Y., Fushimi, H., Sweetman, J., 2013. Effects of inactivated *Enterococcus faecalis* and mannan oligosaccharide and their combination on growth, immunity, and disease protection in rainbow trout. North. Am. Aquac. 75, 416–428.
Roosta, Z., Hoseinifar, S.H., 2016. The effects of crowding stress on some epidermal mucus immune parameters, growth performance and survival rate of Tiger barb (*Pentius tetrazona*). Aquac. Res. 47, 1682–1686.
Ross, N., Firth, K., Wang, A., Burka, J.F., Johnson, S., 2000. Changes in hydrolytic enzyme activities of naive Atlantic salmon *Salmo salar* skin mucus due to infection with the salmon louse *Lepeophtheirus salmonis* and cortisol implantation. Dis. Aquat. Org. 41, 43–51.
Rufchaei, R., Hoseinifar, S.H., Mirzajani, A., Van Doan, H., 2017. Dietary administration of *Pontogammarus maeoticus* extract affects immune responses, stress resistance, feed intake and growth performance of Caspian roach (*Rutilus caspicus*) fingerlings. Fish. Shellfish. Immunol. 63, 196–200.
Safari, R., Hoseinifar, S.H., Nejadmoghadam, S., Jafar, A., 2016. Transciptomic study of mucosal immune, antioxidant and growth related genes and non-specific immune response of common carp (*Cyprinus carpio*) fed dietary Ferula (*Ferula assafoetida*). Fish. Shellfish. Immunol 55, 242–248.
Saitou, N., Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4, 406–425.
Sheikhzadeh, N., Heidarieh, M., Karimi Pashaki, A., Nofouzi, K., Ahrab Farshbafi, M., Akbari, M., 2012. Hilyses®, fermented *Saccharomyces cerevisiae*, enhances the growth performance and skin non-specific immune parameters in rainbow trout (*Oncorhynchus mykiss*). Fish. Shellfish. Immunol 32, 1083–1087.
Siwicki, A.K., Anderson, D.P., 1993. Nonspecific Defense Mechanisms Assay in Fish: II. Potential killing activity of neutrophils and macrophages, lysozyme activity in serum and organs and Total immunoglobulin level in serum. Fish Dis. Diagn. Prev. Methods 1993, 105–112 Olsztyn, Poland.
Soleimani, N., Hoseinifar, S.H., Merrifield, D.L., Barati, M., Abadi, Z.H., 2012. Dietary supplementation of fructooligosaccharide (FOS) improves the innate immune response, stress resistance, digestive enzyme activities and growth performance of Caspian roach (*Rutilus rutilus*) fry. Fish. Shellfish. Immunol 32, 316–321.
Song, S.K., Beck, B.R., Kim, D., Park, J., Kim, J., Kim, H.D., 2014. Prebiotics as immunostimulants in aquaculture: a review. Fish. Shellfish. Immunol. 40, 40–48.
Sriphannam, W., Lumyong, S., Niumsap, P., Ashida, H., Yamamoto, K., Khanongnuch, C., 2012. A selected probiotic strain of *Lactobacillus fermentum* CM33 isolated from breast-fed infants as a potential source of β-galactosidase for prebiotic oligosaccharide synthesis. J. Microbiol. 50, 119–126.
Subramanian, S., MacKinnon, S.L., Ross, N.W., 2007. A comparative study on innate immune parameters in the epidermal mucus of various fish species. Comp. Biochem. Physiol. B: Biochem. Mol. Biol. 148, 256–263.
Sun, Y.Z., Yang, H.L., Marl, R.L., Song, K., Li, J.S., 2011. Effect of *Lactococcus lactis* and *Enterococcus faecium* on growth performance, digestive enzymes and immune response of grouper *Epinephelus coioides*. Aquac. Nutr. 18, 281–289.
Sunyer, J., Tort, L., 1995. Natural hemolytic and bactericidal activities of sea bream *Sparus aurata* serum are affected by the alternative complement pathway. Vet. Immunol. Immunopathol. 45, 333–345.
Suzer, C., Coban, D., Kamaci, H.Q., Saka, S., Firat, K., Otgucuoglu, O., Kucuksari, H., 2008. *Lactobacillus* spp. Bacteria as probiotics in gilthead sea bream (*Sparus aurata*) larvae: effects on growth. Aquaculture. 280, 140–145.
Swain, S.M., Singh, C., Arul, V., 2009. Inhibitory activity of probiotics Streptococcus phocae PI80 and Enterococcus faecium MC13 against vibriosis in shrimp *Penaeus monodon*. World J. Microbiol. Biotechnol. 25 (4), 697–703.
Van Doan, H., Hoseinifar, S.H., Khanongnuch, C., Kanpiengjai, A., Unban, K., Srichaiyo, S., 2018. Host-associated probiotics boosted mucosal and serum immunity, disease resistance and growth performance of Nile tilapia (*Oreochromis niloticus*). Aquaculture. 491, 94–100.
Van Hai, N., 2015. The use of medicinal plants as immunostimulants in aquaculture: a review. Aquaculture. 446, 88–96.
Wang, Y.B., Tian, Z.Q., Yao, J.T., Li, W.F., 2008. Effect of probiotics, *Enteroccus faecium*, on tilapia (*Oreochromis niloticus*) growth performance and immune response. Aquaculture. 277, 203–207.
Wang, W., Sun, J., Liu, C., Xue, Z., 2016. Application of immunostimulants in aquaculture: current knowledge and future perspectives. Aquac. Res. 54, 285–293.
Yanbo, W., Zirong, X., 2006. Effect of probiotics for common carp (*Cyprinus carpio*) based on growth performance and digestive enzyme activities. Anim. Feed Sci. Technol. 127, 283–292.
Ye, J.D., Wang, K., Li, F.D., Sun, Y.Z., 2011. Single or combined effects of fructo- and mannan oligosaccharide supplements and *Bacillus clausii* on the growth, feed utilization, body composition, digestive enzyme activity, innate immune response and lipid metabolism of the Japanese flounder *Paralichthys olivaceus*. Aquac. Nutr. 17, 902–911.
Ziaei-Nejad, S., Rezaei, M.H., Takami, G.A., Lovett, D.L., Mirvaghefi, A.R., Shakouri, M., 2006. The effect of *Bacillus* spp. bacteria used as probiotics on digestive enzyme activity, survival and growth in the Indian white shrimp *Fenneropenaeus indicus*. Aquaculture. 252, 516–524.