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# Comparative study of host-associated and commercial probiotic effects on serum and mucosal immune parameters, intestinal microbiota, digestive enzymes activity and growth performance of roach (*Rutilus rutilus caspicus*) fingerlings

**Running head:** host-associated probiotic versus commercial probiotic

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**Abstract**

The study aimed to isolate host-associated probiotic (HAP) lactic acid bacteria from intestine of adult Caspian roach and compare the efficacy of HAP with a commercially available probiotic strain (*Pediococcus acidilactici*) on the growth and feed utilisation, digestive enzymes and systemic and mucosal immune system of roach fingerling. The HAP strain isolated from roach intestine was *Enterococcus faecium* strain CGMCC1.2136. The experiment was a simple completely randomized design and lasted for eight weeks. Two hundred and seventy fish with an average weight of 12 g randomly distributed into nine tanks. The trial consisted of three treatments with three respective replications. During the experimental period, fish received basal diet without any bacterial supplementation (as the control group), basal diet enriched with $10^8$ CFU $g^{-1}$ HAP or $10^7$ CFU $g^{-1}$ CP. At the end of the experiment, serum immune parameters of those fish fed HAP including alkaline phosphatase activity, total protein content, total immunoglobulin level, lysozyme activity and complement activity (ACH50) were significantly higher that other experimental groups ($P<0.05$). Similarly, dietary supplementation of HAP resulted in better mucosal immune parameters in comparison to control group and commercial probiotic administration ($P<0.05$). Intestinal heterotrophic bacteria and autochthonous LAB counts of those fish fed HAP were significantly higher than other experimental groups at the end of the experiment as well as 15 days seizing probiotic administrations ($P<0.05$). Fish fed with HAP containing diet presented significantly higher amylase, lipase and protease activity in comparison to the CP fed fish and the control group ($P<0.05$). Growth indices of those fish fed HAP were significantly higher than other treatments ($P<0.05$). The highest carcass protein and ash content along with the lowest body moisture content belonged to those fish received HAP ($P<0.05$). In conclusion, the use host-HAP resulted in better immune competence and growth performance and it seems aquaculture sector should probably focus on the development of

probiotics isolated from the cultured species instead of using terrestrial probiotics with greatly different requirements and environmental conditions.

**Keywords:** Non-specific immunity; Roach; Host-associated probiotic; *Enterococcus faecium*

## Introduction

Consumption of fish and seafood is expected to rise by nearly one third in the next few years as a result of growing population, development of lower middle income countries (LMICs) and changing consumption patterns [1, 2]. Aquaculture plays an increasingly important role in satisfying demand of aquatic products, since capture fisheries have remained stable for the last few decades [3]. Global aquaculture production is expected to increase 62% (35 million tons) between 2010 and 2030, with over 90% of that growth taking place in LMICs [4]. However, in order to achieve such growth, problems hindering aquaculture development need to be addressed. Aquatic animal disease is one of the major limiting factors for expansion of aquaculture industry, causing economic losses over US$6 billion per annum [4-6].

Disease prevention and control is often achieved by the use of synthetic drugs, which are administered orally, in baths or injections [7]. However, such practices not only have hazardous effects on the environment, but can also be detrimental to the cultured organisms [8]. Furthermore, their widespread use leads to the emergence of resistance, posing serious threats to the aquaculture sector and global health [9-11]. Vaccination is another alternative for disease management, but commercial vaccines are often too expensive for widespread use by fish farmers, and they are ineffective in multi-agent infections [12]. Therefore, new sustainable alternatives are needed and extensive research has been conducted on the use of food additives (e.g. medicinal plants and probiotics) that act as immunostimulants and decrease disease susceptibility [13-15].

The use of probiotics, living microbial food additives, is especially interesting in aquaculture because besides stimulating the immune system they can also improve growth and feed conversion [16, 17]. A better use of aquaculture resources, which includes limiting fishmeal, is required for the sustainable development of aquaculture [13]. Therefore, the incorporation in probiotics in fish diets could contribute to both better disease management and improvement of feeding efficiency [18]. Traditionally, aquaculture research and development has focused on the use of probiotics (mostly *Bacillus spp.* and lactic acid bacteria [LAB]) isolated from terrestrial sources such as milk and cheese, but recent studies suggest that host-associated microorganisms might perform better [19-22]. Evidence indicates that host-associated probiotics (HAPs) would have better chances to persist in the colonic environment after probiotic withdrawal whilst commercial probiotics might not adapt well in such low pH environments [20].

This study aimed to isolate HAP lactic acid bacteria from intestine of adult Caspian roach and compare the efficacy of HAP with a commercially available probiotic strain (*Pediococcus acidilactici*) on the growth and feed utilisation, digestive enzymes and systemic and mucosal immune system of roach fingerlings. The commercial strain was selected to test efficacy versus HAP as previous studies revealed beneficial effects of this strain on several fish and shellfish strain [23-28]. Culture of Caspian roach is highly valuable in the Caspian Sea since natural populations are currently threatened and programs of restocking have been implemented by local entities such as the Iran Fisheries Organization [29]. Within this context, the management of roach diseases, especially during sensitive life stages (e.g. larvae and fry) is critical and development of suitable probiotics can offer new sustainable solutions.

## 2. Materials and Methods

### 2.1. Screening of host associated LAB from the digestive tract of fish.

Fifteen apparently healthy Caspian roach adult were caught from Gorgan gulf (36.8469° N, 53.9542° E) and transported to laboratory of animal science of GUASNR. The fish starved for 24 hrs and the killed with over-dose of anaesthetic (clove powder). Then, the ventral surfaces of the fish were swabbed with 1% iodine solution before dissection. The intestines from the fish were removed aseptically and homogenized (Potter– Elvehjem Tissue Homogenizer, Cole-Parmer Instrument Company, IL, USA) using 0.9% NaCl solution. The resulting homogenate was used as an inoculum. One ml of the homogenate was spread on sterilized soybean-casein digest agar (tryptone soya agar, TSA) and de Man, Rogosa and Sharpe (MRS) agar plates and incubated at 37°C for 24 hours in duplicate. Colonies with a different morphological appearance were isolated and streaked separately on TSA plates for identification and characterization.

**2.2. Phenotypic, biochemical and molecular characterization of bacterial isolates**

All selected isolates were characterized using phenotypic traits including colony morphology, catalase, Gram staining and spore formation following standard procedures (Johnson and Case, 1995). Further tests were done to screen for the presence of lactic acid bacteria using bacterial movement, fermentation and growth in selective media following the methods of Case and Johnson [30], Harrigan and McCance [31], Harrigan and McCance (1976), Oyewole and Odunfa [32]. The bacterial colonies that were presumed to be lactic acid bacteria were tested of their ability to grow in various temperatures and salinity levels [33], ability to autoaggregate or co-aggregate [34], antagonism against *Aeromonas hydrophila* and *Yersinia ruckeri* [35], tolerance to bile salts and acids [36] and cell surface hydrophobicity [37] following previously published procedures. At last to identify the seleceted colony, total DNA was extracted according to Marmur method [38]. PCR reaction was carried out using 27F and 1492R universal primers (Table 1). The sequencing was done by applied biosystems 3730/3730xL DNA analyzers, (Bioneer, Seoul, Korea) using Sanger method.

125 **2.3. Experimental diets and Fish husbandry**

126 Caspian roach fingerlings were supplied by Sijawal Caspian Sea teleost fish research center.
127 Upon arrival, fish were adopted to experimental condition for 2 weeks. Thereafter,
128 fingerlings (12.0 ± 1.0 g) were stocked in nine 120-L Fiberglas tanks at rate of 30 fish per
129 tank. Considering, the results of our previous study (Tarkhani et al. in press) on the effects of
130 HA *Enterococcus faecium* strain CGMCC1.2136 isolated from adult roach on immunity,
131 digestive function and growth of the fish fingerlings, we selected the optimum dose ($10^8$ CFU
132 $g^{-1}$) compare the efficacy of HAP with a commercial strain (Bactocel, *P. acidilactici*) which
133 was tested before and its beneficial effects was well-documented. To that end, a basal diet
134 was prepared as described in our previous study [39]. The proximate chemical composition of
135 the basal commercial diet is presented in Table 1. The control treatment was fed with basal
136 diet and for other two groups (i.e. HAP and CP) basal diet was supplemented with $10^8$ CFU
137 $g^{-1}$ of HA *Enterococcus faecium* strain CGMCC1.2136 and $10^7$ CFU $g^{-1}$ *P. acidilactici*. The
138 experimental diets were prepared as described by [40]. The level of CP (commercial
139 probiotic) was selected based on the results of previous studies. The feeding trial lasted for 8
140 weeks and during this period fish in each tank were fed on corresponded diet at the rate of 3%
141 of their respective body weight (adjusted based on regular biometry). The culture system was
142 static aerated water with daily water exchange to ensure desirable water quality. The
143 photoperiod of 15 hours Light to 9 hours Dark was employed and all water quality criteria
144 including pH= 7.6-7.9, DO= 8.5±0.12 ppm and temperature= 17.3±0.2° C were monitored
145 every day and were well within the optimum prerequisites for the fish.

146 **2.4. Immunological analyses**

147 After an eight-week feeding trial, nine fish (three per replicate) were randomly taken from
148 each treatment and anesthetized using clove oil. Then, blood samples were obtained from the
149 caudal vein by means of 5 ml non-heparinized syringes. Blood samples were centrifuged

(Heraeus Labofuge 400) at 1600 g for 10 min to obtain sera. The collected sera were kept at -80ºC until analysis.

Using a lysozyme sensitive bacterium (*Micrococcus lysodeikticus*) in lyophilized form, the lysozyme activity of serum samples was measured according to the standard protocol suggested by Ellis [41]. The total protein content of serum samples were determined according to the Biuret method by means of a commercially available kit (Pars-Azmun Co., Tehran, Iran). The protocol suggested by Siwicki and Anderson [42] was followed to determine the total immunoglobulin (IgM) level in serum samples. The method was based on determination of protein level before and after participating down the immunoglobulin molecules using polyethylene glycol (Sigma). The rabbit RBCs were used to assay Alternative complement activity (ACH50) as described in our previous publication [43]. The serum volume producing 50% haemolysis (ACH50) was measured and used to calculate the complement activity. Finally, Alkaline phosphatase activity of serum samples were 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).

**2.5. Intestinal microbiota analysis**

At the end of feeding trial and 15 days after seizing probiotic supplemented diets; the total viable heterotrophic aerobic bacteria and lactic acid bacteria (LAB) counts were determined in intestinal microbiota. Nine fish were randomly selected from each treatment (three per replicate) and the intestine samples were collected and prepared for bacteria culture as described in our previous publication [44]. The plate count agar (PCA) (Merck, Germany) and MRS agar media (Merck, Germany) were used to determine the total count and LAB count, respectively. After five days incubation at room temperature (25 ºC) the colonies were counted from statistically viable plates (i.e. plates containing 30-300 colonies) [25].

### 2.6. Digestive enzymes activity assay

At the end of feeding, fish were starved for 24 h and then nine specimens were randomly sampled to study the effects of HAP or CP on digestive enzymes activity including alkaline protease, lipase and amylase. Fish were anesthetized by clove oil and euthanized by severing the spinal cord. The intestine samples were obtained and washed by cold normal saline solution and stored at -80 ºC until use. All sampling procedures were carried out on ice-cold trays. The crude enzyme extracts were prepared as described in our previous publication [45]. Alkaline protease activity was determined according to GARCÍA□CARREÑO and HAARD [46] using 2% Azocasein solution in Tris-HCl, pH=7.5 as the substrate and the activity was reported as U per mg protein per min. Hydrolysis of ρ-nitrophenyl myristate, as the substrate, was recorded at 405 nm to determine the specific activity of lipase as described in our previous work [45]. Using starch as substrate, α-amylase activity was determined based on the method developed by Bernfeld, [30]. The specific activity of α-amylase was expressed as μmol of maltose released per mg protein per min at 25 °C.

### 2.7. Growth performance and body composition

At the end of feeding trial all fish were weighed to measure the growth performance parameters as mentioned below. Also, five fish were sampled and stored -20˚C to analyse body composition according Yarahmadi et al. [47].

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

*Specific growth rate* (%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* = g feed intake / g live weight gain

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

### 2.8. Statistical analysis

Before analysis the standard normality test (i.e. Kolmogorov-Smirnove) was done to confirm normality of dataset. Then, One-way ANOVA was used to elucidate if there were significant differences ($P<0.05$) between treatments followed by Tukey's HSD test. SPSS software ver. 16 was used to perform the statistical analyses

## 3. Results

### 3.1. Characterization of host-associated probiotic candidates

A total of forty-eight isolates were initially characterized from the gastrointestinal tract of Caspian roach juveniles (Supplemental Tables 1-9). From this number of isolates, an isolates were selected based on its phenotypic and biochemical characteristics (Table 3). The isolate designated as L9 was bacilli, coccus in shape, Gram positive, do not to possess catalase activity, non-spore formers and non-motile. It could grow in both MacConkey and Stat agar media, have wide tolerance to temperature and salinity levels. The bacterial isolate exhibited auto-aggregation and co-aggregation activities and have the ability to inhibit the growth of *A. hydrophila* and *Y. ruckeri* in vitro. It was able to survive when exposed to acid and bile salts and showed cell surface hydrophobicity. The molecular studies and sequencing of the 16s RNA of the isolate showed that it was identified as putative *Enterococcus faecium* strain CGMCC1.2136.

### 3.2. Serum immune parameters

Levels of alkaline phosphatase activity were significantly higher ($P < 0.05$) in fish fed with the roach-associated probiotic than control and fish fed with the commercial probiotic (Figure 1A). No significant difference ($P > 0.05$) was observed between the levels of total protein between the fish from the three treatments (Figure 1B). Fish fed with the diet enriched in host-associated *E. faecium* displayed significantly higher ($P < 0.05$) total immunoglobulin levels, whilst control and fish fed with the diet enriched in the commercial probiotic did not show a significant difference between them ($P > 0.05$) (Figure 1C). Finally, lysozyme activity

and complement activity (ACH50) were both the highest ($P < 0.05$) in fish fed with the roach-associated probiotic and lowest ($P < 0.05$) in the fish fed with the commercial probiotic (Figure 1D, 1E).

### 3.3. Mucosal immune parameters

Fish fed with the diet enriched in *E. faecium* isolated from adult roach presented significantly higher ($P < 0.05$) mucosal alkaline phosphatase activity, total protein, total immunoglobulin and protease levels than control fish and fish fed with the commercial probiotic (Figure 2A, 2B, 2C, 2D). Lysozyme activity was the highest ($P < 0.05$) in fish fed with the enriched diet with the autochthonous probiotic and the lowest ($P < 0.05$) in fish fed with the diet enriched with the commercial probiotic (Figure 2E).

### 3.4. Intestinal microbiota

Figure 3A and 3B represents the effects of feeding roach fingerling with HAP and CP on total autochthonous intestinal heterotrophic bacteria and autochthonous LAB, respectively. evaluation of total count of bacteria at the end of feeding trial revealed significantly higher bacteria load in both groups fed with HAP and CP compared control treatment ($P < 0.05$) (Fig. 3A). Regarding LAB count, although no LAB was isolated from statistically statistically viable plates, the LAB levels in HAP and CP group were $3.48 \pm 0.04$ log CFU $g^{-1}$ and $3.48 \pm 0.04$ log CFU $g^{-1}$, respectively (Fig. 3B). The highest LAB count was observed in HAP group which was significantly higher than other groups ($P < 0.05$). The results of microbiological studies 15 days after seizing probiotic administration in diet revealed the same trend as the last day of the trial (4A and 4B). Indeed, no drastic changes were noticed in LAB count both in HAP and CP groups and the highest LAB count were remained to be in HAP treatment.

### 3.5. Digestive enzymes activity

Fish fed with a diet enriched in host-associated *E. faecium* presented significantly higher levels of amylase, lipase and protease than the fish fed with the commercial probiotic and the

control ($P < 0.05$, Figure 5A, 5B, 5C). Fish fed with the diet enriched in the commercial probiotic displayed significantly higher levels of amylase than the control ($P < 0.05$), but no significant different was observed between the levels of lipase and protease (Figure 5A, 5B, 5C).

### 3.6. Growth performance

Juvenile roach fed with a diet enriched in host-associated *E. faecium* during 8 weeks displayed significantly higher ($P < 0.05$) final weight (FW), weight gain (WG) and specific growth rate (SGR) than control group and the fish fed with the diet enriched with the commercial probiotic (Table 4). Feed conversion ratio (FCR) was significantly lower ($P < 0.05$) in fish fed with the host-associated bacteria enriched diet, whilst control and fish fed with the diet containing the commercial probiotic did not display significant differences. All fish from the three treatments survived the experiment (Table 4).

### 3.7. Carcass composition

Approximate analysis of fish carcasses fed with different diets revealed that fish fed with the enriched diet in roach-associated *E. faecium* presented the highest protein content, whilst fish fed with the commercial probiotic presented the lowest ($P < 0.05$, Table 5). Fat content did not differ significantly amongst the treatments ($P < 0.05$). The lowest moisture content was observed in fish fed with the host-associated probiotic, whilst the highest was found in fish fed with the diet enriched in the commercial probiotic ($P > 0.05$, Table 5). Finally, the highest percentage of carcass ash was found in the fish fed with the roach-associated probiotic ($P > 0.05$), whereas the fish fed with the commercial probiotic presented the lowest (Table 5).

## 4. Discussion

Multicellular organisms harbour complex microbial communities that have co-evolved with the host during millions of years and have developed specific functions [48]. The gut microbiota plays a critical role in modulating host's physiology, affecting host behaviour,

regulating feeding, influencing digestive and metabolic processes and modulating immune responses [48-50]. The functions of the gut microbiota depend on the composition of microbes present, which can be partially shaped by environmental parameters or dietary intakes [51-54].

Probiotic administration can modulate gut composition and it has been shown to display numerous beneficial effects on fish such as improved growth and feed utilization, enhancement of immune function and resistance to pathogens [14, 55, 56]. However, research advances that bring light to the importance of host-associated bacteria, question the pertinence of using commercial probiotics isolated from very different environments such as milk [21].

The innate immune system is the first line defence mechanism in fish and thus immunostimulants are often used as prophylaxis treatment to decrease animal susceptibility to infections [57]. Oral probiotic administration in fish can enhance the innate immune system, either via the interaction with immune cells (e.g. phagocytes, leukocytes) or by increasing the humoral immune system [18, 58]. In this study we found that oral administration of *E. faecium* isolated from adult roach increased the mucosal and serum immune parameters of roach fingerlings. Alkaline phosphatase (ALP) is an important lysozomal enzyme with a putative protective role in the initial stage of wound healing and antibacterial activity [59]. Administration of host-associated probiotic increased significantly ALP activity in roach fingerlings. Similar results were observed after the dietary administration of *Lactobacillus acidophilus* in black swordtail (*Xiphophorus helleri*) [60].

Increases in serum and mucosal proteins are believed to be associated to the increase of immune-related proteins (e.g. proteases, lysozymes, lectins and globulins) and thus to be related with a more active innate immune system [61, 62]. Our results found significant higher protein levels in mucus from fish fed with host-associated probiotics; however, we did

not observe a significant difference in serum total protein between fish from the different treatments. Increased mucus levels after probiotic administration were found in *X. helleri* and rainbow trout (*Oncorhynchus mykiss*) and it was suggested they might be related to higher mucosal antibacterial activity [60, 63]. Immunoglobulins are natural antibodies that play a key role in both innate and adaptive immunity, producing specific antibody responses against various antigens [64]. Higher levels of total immunoglobulin have been previously reported in fish after dietary supplementation with probiotics [23, 65-67]. In this study we observed significantly higher levels in fish fed with the host-associated *E. faecium* strain, however no difference was observed between the control fish and fish fed with the commercial probiotic, indicating the better immunostimulant efficacy of the host-derived probiotic.

Lysozyme is a bacteriolytic enzyme, which can also trigger other immune responses such as the complement system and phagocytic cells [68]. Therefore the higher lysozyme levels observed in fish fed with the host-associated probiotic suggest an enhanced immune system in these fish and better resistance against infections. Lysozyme is a widely used measure of innate immunity, and higher lysozyme levels after probiotic administration have been often reported (e.g. [23, 69-71]). However, it's worth remarking that roach fingerlings fed with the commercial probiotic displayed significantly lower lysozyme levels than control, suggesting that administration of exogenous bacteria might sometimes display negative effects, maybe by the replacement of the natural beneficial microbiota. Although, such phenomenon should be further studied.

Complement plays a major role in clearing pathogens as well as in the development of adaptive immune response [72]. We observed that fish fed with roach-associated *E. faecium* displayed the highest alternative complement activity, whilst fish fed with the commercial probiotic showed a reduction of the $ACH_{50}$ compared to control fish. These findings agree with previous studies that find an increase in the activity of alternative complement after

probiotic administration during short feeding periods (< 40 days) (e.g. [23, 24, 73-75]). However, some studies found that when probiotic was administered during longer periods (60 days), complement system was activated during the initial feeding period (30 days) and decreased to control levels after [74, 76].

In this study, we have compared the efficacy of a host-derived probiotic strain (*E. faecium*) and a commercial strain on the growth and feed utilisation, digestive enzyme activity and systemic and mucosal immune parameters of roach fingerlings. Our results show that fish fed with a diet enriched with the host-associated probiotic presented higher growth and better feeding efficiency, higher quality of carcasses (protein level), higher digestive enzyme levels and higher systemic and mucosal immune parameters than control fish and fish fed with a diet enriched with a commercial probiotic strain, indicating the importance of using host-specific probiotic strains.

The intestinal of fish harbour numerous bacterial species which form intestinal microbiota. Although constitute a minor part of intestinal microbiota in fish species, several beneficial effects attributed to LAB bacteria. Therefore, there was increasing interests toward increasing their number in intestinal microbiota using different pre and probiotics. Previous studies, demonstrated elevation of LAB counts in intestine following oral administration of probiotics [25, 60, 77, 78]. Likewise, our findings showed significant increase of LAB in both HAP and CP fed groups compared control treatment. Also, another finding of our study was no significant alteration of LAB levels in intestine 15 days after stopping probiotic administration (feeding all treatments with basal diet). This possibly shows that both HAP and CP could have stable colonization in roach fingerlings intestinal microbiota. Also, LAB levels in HAP fed fish intestinal microbiota was significant higher than others, hint that the condition was more favourite for HAP rather than CP. This was supported by the more beneficial effects observed in measured parameters of fish in HAP treatment.

Higher fish growth and feeding efficiency after probiotic administration has been reported in several studies (e.g. [79-81]. Probiotics can also promote short-chain fatty acid production (e.g. acetate, propionate and butyrate), which can modify intestinal morphology, increasing villus length and enabling higher nutrient absorption [82-84]. Use of diet supplements that improve feeding efficiency and digestion processes have a huge potential in the viability of using alternative protein sources such as plants and will become more necessary as the pressure for reducing fishmeal increases [85, 86]. The present results revealed that supplementation of probiotic in diet increase digestive enzymes activity. Interestingly, fish fed HAP probiotic had significantly higher digestive enzymes such as amylases and proteases that are crucial for detoxifying and denaturing indigestible components in feed [17]. This finding is in line with previous reports regarding HAP administration in aquaculture [87]. The modulation of digestive enzymes activity after probiotic utilization has been ascribed to to production of extracellular enzymes by probiotics bacteria after successful adhesion and colonization [17, 88]. The present finding clearly shows that HAP is more effective to improve digestive capacity of roach.

In conclusion, we showed that dietary administration of *E. faecium* isolated from adult roach was significantly more effective in promoting growth, feeding efficiency, secretion of digestive enzymes and enhancing the mucosal and systemic immune systems than the commercial strain (*P. acidilactici*) in roach fingerlings. Furthermore, the commercial strain suppressed some immune parameters such as lysozyme or complement activity, suggesting it might display antagonistic effects on native roach microbiota. Altogether, these results suggest that the use host-associated probiotics have a better performance and that aquaculture sector should probably focus on the development of probiotics isolated from the cultured species instead of using terrestrial probiotics whose requirements and environmental conditions greatly differ.

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Table 1. The sequence of forward and reverse primer used to identify the strain

| Primer name | **Primer Sequence (5' →3')** |
|---|---|
| 27F | AGAGTTTGATCMTGGCTCAG |
| 1492R | GGTTACCTTGTTACGACTT |

Table 2. Composition of the basal diet used throughout the experiment

| Proximate analysis (%) | |
|---|---|
| Dry matter | 92.6 |
| Crude protein | 38.45 |
| Crude lipid | 9.78 |
| Ash | 10.12 |
| Crude Energy (cal.$g^{-1}$) | 4389.35 |

Table 3. Phenotypic and biochemical characteristics of probiotic candidate isolated from the gut of Caspian roach juveniles.

| **Characteristics** | **Isolate L9** |
|---|---|
| Morphology | bacillus, coccus |
| Catalase activity | - |
| Gram-staining | + |
| Spore formation | - |
| Movement | - |
| Fermentation activity | + |
| Growth in Med.α (MacConkey Medium) | + |
| Growth in Med.β (Stat Agar Medium) | + |
| Growth at 4°C | + |
| 10°C | + |
| 20°C | + |
| 30°C | + |
| Growth at 4 ppt | + |
| 8 ppt | + |
| 12 ppt | + |
| 30 ppt | + |
| Autoaggregation | 9.05 |
| Coaggregation | 17.172 |
| Inhibition of *Aeromonas hydrophila* (diameter in mm) | 12.52 |
| Inhibition of *Yersinia ruckeri* (diameter in mm) | 12.28 |
| Tolerance to acid | 63.64 |
| Tolerance to bile salts | 85.71 |
| Cell surface hydrophobicity (%) | 66.55 |

Table 4. The effects of dietary administration of host-associated *Enterococcus faecium* strain CGMCC1.2136 ($10^7$ CFU $g^{-1}$) and commercial probiotic *P. acidilactici* ($10^7$ CFU $g^{-1}$) on growth performance, feed utilisation and survival rate of Caspian roach fingerling.

| | treatments | | |
|---|---|---|---|
| | 0.00 (Control) | CP | HAP |
| Initial weight (g) | 12.779 ± 0.06 [a] | 12.863 ± 0.05 [a] | 12.808 ± 0.03 [a] |
| Final weight (g) | 24.577 ± 0.42 [b] | 24.666 ± 0.13 [b] | 28.111 ± 0.16 [a] |
| WG (g) | 11.798 ± 0.46 [b] | 11.803 ± 0.81 [b] | 15.302 ± 0.19 [a] |
| WG (%) | 92.333 ± 3.92 [b] | 91.755 ± 6.25 [b] | 119.472 ± 1.77 [a] |
| SGR (% $day^{-1}$) | 1.089 ± 0.03 [b] | 1.084 ± 0.05 [b] | 1.31 ± 0.01 [a] |
| FCR | 2.488 ± 0.09 [a] | 2.492 ± 0.16 [a] | 1.917 ± 0.02 [b] |
| Survival (%) | 100 | 100 | 100 |

Values are mean ± S.D and in each row with different letters denote significant differences ($P < 0.05$).

Table 5. The effects of dietary administration of host-associated *Enterococcus faecium* strain CGMCC1.2136 ($10^7$ CFU $g^{-1}$) and commercial probiotic *P. acidilactici* ($10^7$ CFU $g^{-1}$) on Caracas composition of Caspian roach fingerling.

| | treatments | | |
|---|---|---|---|
| | 0.00 (C) | CP | HAP |
| Protein | 14.227 ± 0.07 [b] | 13.21 ± 0.04 [c] | 15.65 ± 0.16 [a] |
| Fat | 7.433 ± 0.1 [a] | 7.44 ± 0.33 [a] | 7.467 ± 0.25 [a] |
| Moisture | 75.113 ± 0.2 [b] | 76.26 ± 0.19 [a] | 72.773 ± 0.3 [c] |
| Ash | 3.163 ± 0.08 [ab] | 3.033 ± 0.13 [b] | 3.987 ± 0.31 [a] |

Values are mean ± S.D and in each row with different letters denote significant differences ($P < 0.05$).

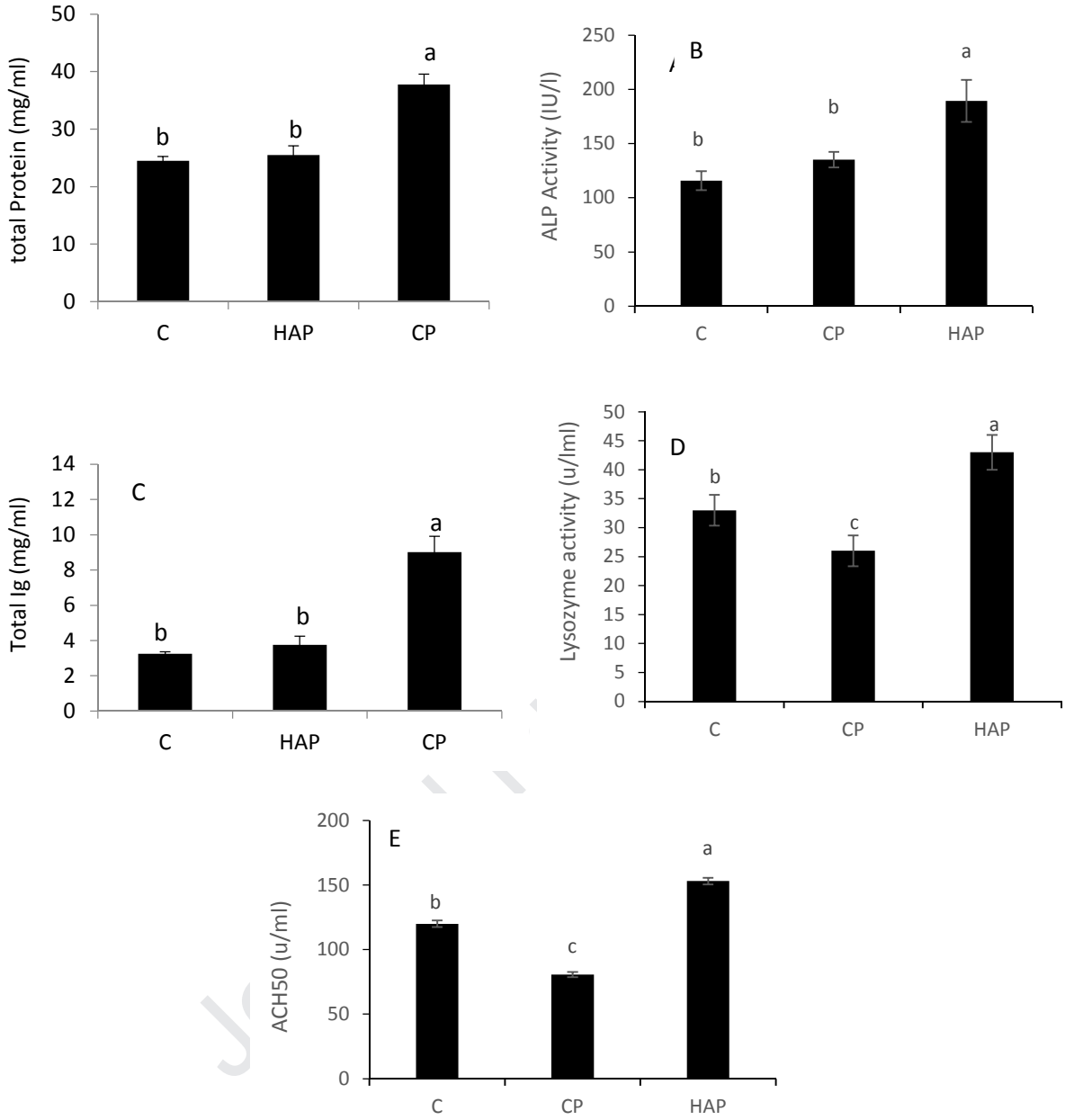


Figure 1. Serum Alkaline phosphatase activity (A), total protein (B), total Ig (C), Lysozyme (D) and complement activity (E) of Caspian roach fingerlings (n=9) fed host-associated (HAP) *Enterococcus faecium* strain CGMCC1.2136 ($10^7$ CFU $g^{-1}$) and commercial probiotic (CP) *P. acidilactici* ($10^7$ CFU $g^{-1}$) for 8 weeks. Bars assigned with different superscripts are significantly different ($P < 0.05$); Values are presented as the mean ± S.D.

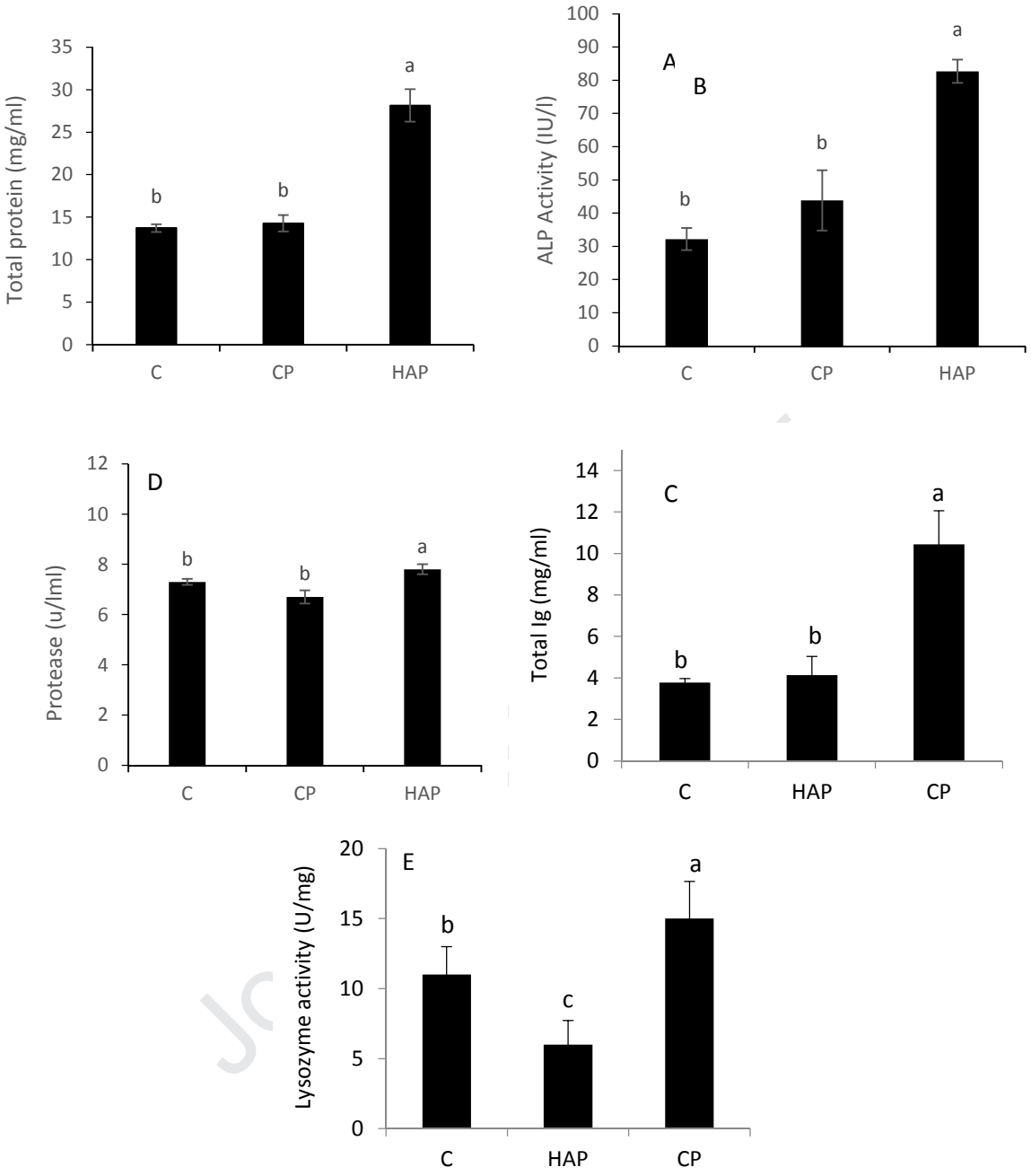


Figure 2. Skin mucus Alkaline phosphatase activity (A), total protein (B), total Ig (C), protease (D) and Lysozyme activity (E) of Caspian roach fingerlings (n=9) fed host-associated (HAP) *Enterococcus faecium* strain CGMCC1.2136 ($10^7$ CFU $g^{-1}$) and commercial probiotic (CP) *P. acidilactici* ($10^7$ CFU $g^{-1}$) for 8 weeks. Bars assigned with different superscripts are significantly different ($P < 0.05$); Values are presented as the mean ± S.D.

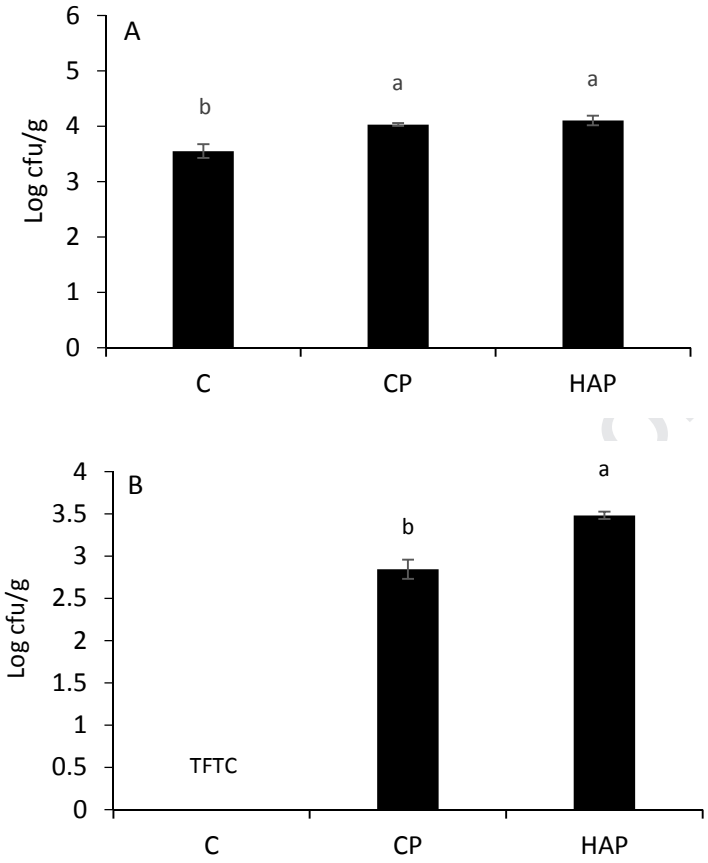


Figure 3. Total culturable autochthonous bacterial (A) and autochthonous lactic acid bacteria (LAB) (B) levels (log CFU $g^{-1}$ intestine) of Caspian roach fingerlings (n=9) fed host-associated (HAP) *Enterococcus faecium* strain CGMCC1.2136 ($10^7$ CFU $g^{-1}$) and commercial probiotic (CP) *P. acidilactici* ($10^7$ CFU $g^{-1}$) for 8 weeks. Bars assigned with different superscripts are significantly different ($P < 0.05$); Values are presented as the mean ± S.D.

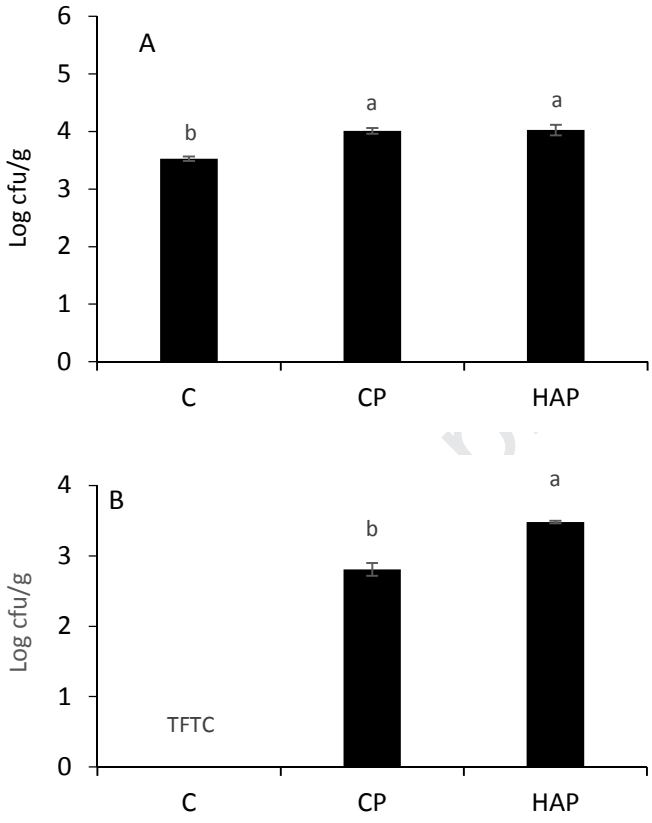


Figure 4. Total culturable autochthonous bacterial (A) and autochthonous lactic acid bacteria (LAB) (B) levels (log CFU $g^{-1}$ intestine) in Caspian roach fingerlings (n=9) 15 days after seizing probiotic administration and reverting to basal diet. Bars assigned with different superscripts are significantly different ($P < 0.05$); Values are presented as the mean ± S.D.

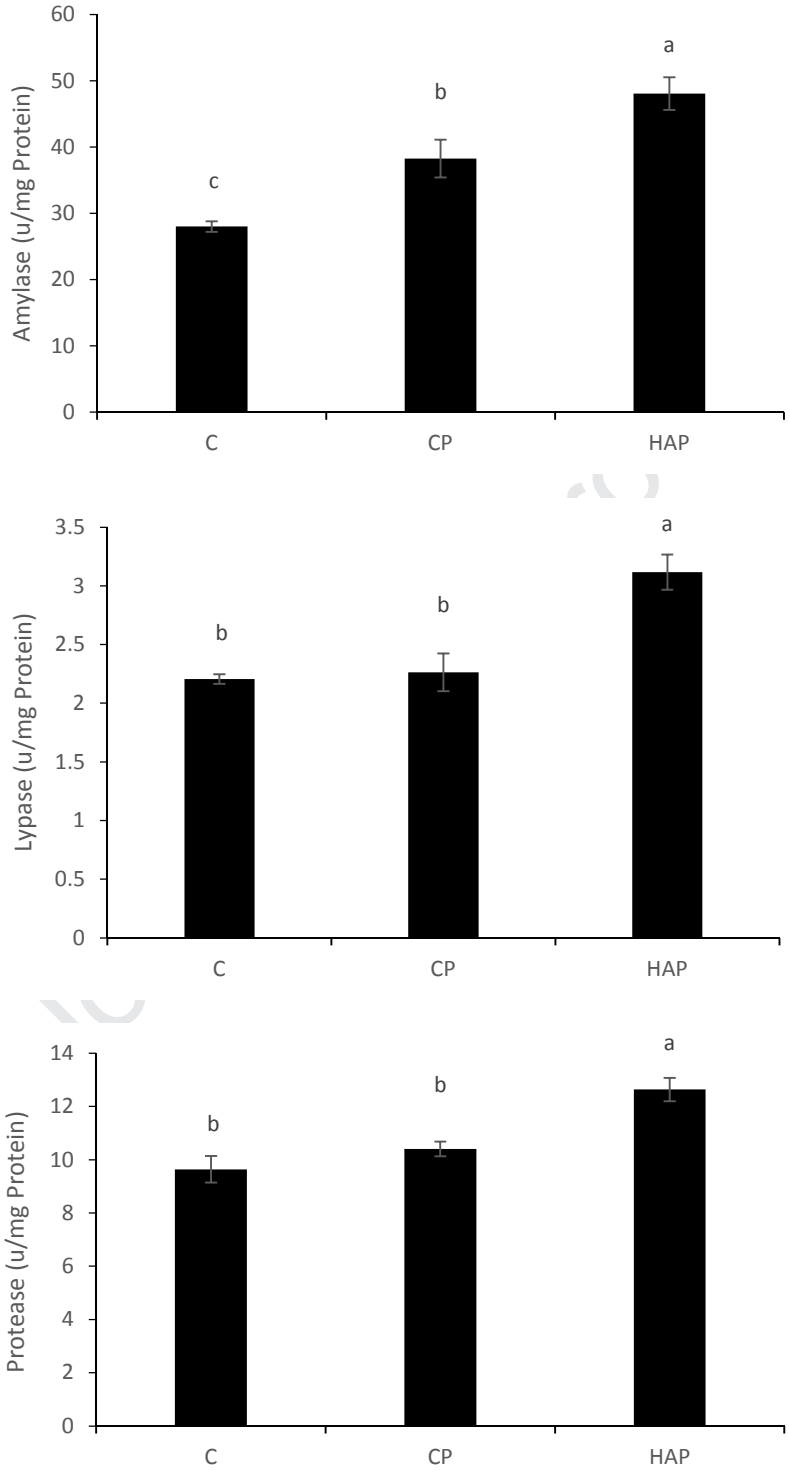


Figure 5. Digestive enzymes activity of Caspian roach fingerlings (n=9) fed host-associated (HAP) *Enterococcus faecium* strain CGMCC1.2136 ($10^7$ CFU $g^{-1}$) and commercial probiotic (CP) *P. acidilactici* ($10^7$ CFU $g^{-1}$) for 8 weeks. Bars assigned with different superscripts are significantly different ($P < 0.05$); Values are presented as the mean ± S.D.

- HAP fed roach showed remarkably increased serum and mucosal immune parameters
- Dietary HAP was more effective than CP in case of modulating digestive enzymes activity.
- Growth performance of fish fed HAP was notably higher than those of CP and control
- LAB count was significantly higher in intestinal microbiota of HAP fed roach.