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ENTEROCOCCUS FAECIUM SUPPLEMENTATION: IMPACTS ON GROWTH AND HEALTH OF PIKEPERCH (*SANDER LUCIOPERCA*)

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Abstract

Sustainable development of aquaculture requires exploring strategies to enhance fish growth and welfare. This study assessed the probiotic potential of *Enterococcus faecium* on growth, immunity, and gut microbiota of farmed *Sander lucioperca* (Gilan, Iran). For this purpose, 180 fish (14.5±0.5 g) were distributed into 12 tanks and fed 4% of their biomass with four experimental diets, including a basal diet (C), basal diet with kanamycin aesculin azide (CP), and basal diets supplemented with 10^{10} (EF1) and 10^{8} (EF2) CFU/g *E. faecium* for 60 days. Results showed significantly higher weight gain and specific growth rate values in fish from EF1 and EF2 groups compared to other treatments (P<0.05). In addition, the feed conversion ratio in EF1 (0.89±0.05) and EF2 (0.83±0.07) was significantly lower than the control group (1.56±0.05), indicating higher feed utilization efficiency in probiotic-fed fish (P<0.05). Regarding serum biochemical parameters, no significant changes were observed for cholesterol, triglyceride, and albumin levels, while total protein and glucose levels were highest in EF1 and EF2 treatments (P<0.05). Fish from EF1 and EF2 exhibited significantly higher lysozyme activity, IgM level, and complement activity (C3 and C4) compared to other treatments (P<0.05). In addition, significantly lower ALP (alkaline phosphatase), ALT (alanine transaminase), and AST (aspartate aminotransferase) activities were observed in EF2 treatments. Finally, the total number of lactic acid bacteria (LAB) in the intestinal tract of fish from the EF1 and EF2 groups was greater than that in the other groups. Along with a significant increase in the total bacterial count and LAB in the gut, it is concluded that *E. faecium* at 10^{10} CFU/g diet can improve the growth performance and immune parameters of *S. lucioperca*.

Key words: aquaculture, gut microbiota, autochthonous probiotics, *Enterococcus faecium*, *Sander lucioperca*

It is generally accepted that fish are more robust and naturally more resistant to infections if fed proper amounts of a well-balanced diet that meets the species-specific nutritional requirements. From decades of knowledge and experience in fish nutrition, it is known that the administration of feed additives can further enhance fish's immune system and growth performance (Adel et al., 2024; Ahmadniaye Motlagh et al., 2023; Arun et al., 2023; Hossain et al., 2023; Wuertz et al., 2021).

The gastrointestinal tract (GIT) of fish fulfills numerous pivotal functions, encompassing digestion and absorption of feedstuff, maintaining water and electrolyte equilibrium, endocrine modulation of digestive and metabolic functions, and immunological defense mechanisms (Buddington et al., 1997). The GIT harbors diverse and intricate microbial communities known as microbiota, which exert evident influences on the health and physiological performance of the host organism (Pérez-Pascual et al., 2021; Vadstein et al., 2013; Wuertz et al., 2021). Therefore, probiotics, defined as live or inactivated microbial components with beneficial effects on fish, have been utilized to augment growth performance, boost health, and enhance disease resistance by modulating the composition of gut microbiota in favor of beneficial microorganisms (El-Saadony et al., 2021; Guardiola et al., 2016; Hoseinifar et al., 2015; Lauzon and Ringø, 2012; Merrifield and Carnevali, 2014; Mirbakhsh et al., 2023; Morshedi et al., 2021; Van Doan et al., 2020; Zorriehzahra et al., 2016). In broad terms, probiotics can stimulate the immune system and regulate the composition of the microbiota (El-Saadony et al., 2021; Fuller, 1989). As of present, the primary probiotics employed in aquaculture predominantly consist of microorganisms such as *Saccharomyces*, *Lactobacillus*, *Bacillus*, *Clostridium, Enterococcus*, *Shewanella*, *Leuconostoc*, *Lactococcus*, *Carnobacterium*, and *Aeromonas* (El-Saadony et al., 2021; Zorriehzahra et al., 2016).

Lactic acid bacteria (LAB) are gram-positive, acid-tolerant, generally non-sporulating, anaerobic, either rod- or cocci-shaped, and known for their probiotic potentials in the intestinal tract of healthy fish (Ringø et al., 2020). *Enterococci* are gram-positive, facultative anaerobic bacteria known to have a low pathogenicity. Recently, *Ente-*

rococcus faecium, a lactic acid bacterium present in the intestinal microflora of many animals and humans, has been suggested as a promising probiotic in aquaculture (Cheriet et al., 2023). However, previous reports have shown that *E. faecium* has been used in animal nutrition as a probiotic, especially in farmed aquatic species (Sun et al., 2012). In addition, Tilwani et al. (2022) have tested 10^6, 10^9, and 10^{12} CFU/g diet of *E. faecium* MC-5 in Indian major carp (*Cirrhinus mrigala*), where 10^{12} CFU/g diet significantly affected growth performance, immune responses and resistance against *Aeromonas hydrophila*. Similar results have been reported in *Arapaima gigas* fed a diet supplemented with 10^8 CFU/g *E. faecium* (da Costa Sousa et al., 2019). It has been suggested that autochthonous probiotics are favored by species-specific driven selection and thus have a better chance of colonizing the gut of the same species (Hoseinifar et al., 2024).

Pikeperch (*Sander lucioperca*) is a major freshwater fish species with substantial economic potential in the Caspian Sea region, and its production has increased in recent years (Falahatkar et al., 2018). In addition, pikeperch is considered a promising fish species in European aquaculture (Policar et al., 2013). Pikeperch can be reared under high-density conditions with artificial feed and is favored by sport anglers (Pvnička and Rybař, 2001). According to the Iranian Fisheries Organization, over 3.5 million pikeperch fingerlings are produced and released for restocking and stock enhancement purposes (the report is accessible in Persian at https://www.fisheries.ir/site/vahed_with_shakhes_v.aspx?uc=50746). To our knowledge, limited information is available regarding the effects of autochthonous probiotics in *S. lucioperca*. Thus, in this experiment, we isolated and identified the bacterium *E. faecium* from *S. lucioperca* and evaluated its probiotic potential *in vivo* on an array of physiological aspects of the same fish species.

Material and methods

Bacterial suspensions

The bacterium was isolated from *S. lucioperca* (Gilan, Iran), biochemically characterized and molecularly identified. To prepare bacterial suspensions, aliquots of 50 µl of probiotic suspension were inoculated into 20 ml of kanamycin aesculin azide broth (KB) and TSB medium and incubated at 30°C for 24 hours. The suspension was then added to 500 ml of KB and TSB medium and incubated at 30°C for 48 hours. After bacterial growth, the suspension was centrifuged at 4000 rpm for 10 minutes, and the resulting pellet was resuspended in sterile physiological saline solution to reach a density of 10^8 and 10^{10} CFU/g. The bacterial counts were determined on nutrient agar plates.

Experimental diets

To date, no feed has been formulated specifically for *S. lucioperca*, and thus, often, commercial salmonid feed is used in studies that result in varying growth performance and survival rates. Since gammarid is a part of the *S. lucioperca* natural diet in its natural habitat, the basal diet was formulated (a modified rainbow trout diet formulation) with gammarid powder and pelletized according to standard procedures to make the artificial diets more acceptable for the fish. The fish were allowed to adapt to experimental conditions for 2 weeks, during which fish were fed the basal diet until apparent satiation. The proximate composition of diets for crude protein, lipid, and ash were measured according to standard methods of AOAC (AOAC, 2000). In brief, protein content was measured by Kjeldahl method (6.25 × nitrogen content) (Foss, Sweden). Moisture content was determined by oven-drying to constant weight at 105°C for diet and at 70°C for fish. Lipid content was measured by the Soxhlet method (Foss, Sweden). Ash was determined by a muffle furnace at 550°C for 6 h.

The basal diet was provided as the control diet, and the bacterial suspensions (10^8 and 10^{10} CFU/g diet) were sprayed onto and mixed with the basal diet using a mixer to prepare experimental diets. The diets were air-dried using an air blower at 30°C under sterile conditions for 2 h and stored at 4°C until use. The number of *E. faecium* in the diets was monitored weekly, and the same diets were prepared weekly. In brief, each gram of fish feed was sprayed with bacterial suspension and incubated at 25°C for 1.5 h in a dry incubator. Samples were taken from various parts of the feed, and the bacterial counts were determined on kanamycin aesculin azide agar (KA) and TSA agar. To determine the number of bacteria per gram of food, sterile PBS solutions with dilutions of 10^{-1}, 10^{-2}, and 10^{-3} and bacterial counts were performed on MRSA, KA, and agar plates.

Table 1. Diet formulation and proximate composition of the basal diet

Ingredients	Amount in %
Fish meal	40
Gammarid powder	20
Soybean meal	7
Maize gluten	6
Wheat gluten	5
Wheat	7
Fish oil	11
Mineral premix[1]	2
Vitamin premix[2]	1
Antioxidant	0.5
Anti-fungi	0.5
Proximate composition (% dry matter basis)	
Crude protein	44.8
Crude lipid	22.5
Ash	11.2
Moisture	7.6

[1]Mineral premix contains (per kg) calcium phosphate 397 g; calcium lactate 327 g; ferrous sulfate 25 g; magnesium sulfate 137 g; potassium chloride 50 g; sodium chloride 60 g; potassium iodide 150 mg; copper sulfate 780 mg; manganese oxide 800 mg; cobalt carbonate 100 mg; zinc oxide 1.5 g; sodium selenite 20 mg. [2]Vitamin premix contains (per g) vitamin A: 50,000 IU, vitamin D_3: 10 IU, vitamin E: 130 g, vitamin K_3: 10 g, vitamin B_1: 10 g, vitamin B_2: 25 g, vitamin B_6: 16 g, vitamin B_{12}: 100 mg, niacin: 200 mg, pantothenic acid: 56 g, folic acid: 8 g, biotin: 500 mg, antioxidant: 0.2 g, anti-cake: 20 g.

Experimental design

The experiment was conducted in a randomized design, including four dietary treatments in triplicates at Inland Waters Aquaculture Research Center (Anzali, Iran). The *S. lucioperca* used in this experiment were procured from a private local farm (Siahkal, Gilan, Iran). The fish were allowed to acclimatize to the experimental conditions for two weeks in 500 L fiberglass tanks, during which fish were fed the formulated basal diet *ad libitum* three times a day. After adaptation, fish (n=180) were bulk weighed (14.5±0.5 g) and each 300-L tank (fiberglass) was stocked with 15 fish (~0.9 kg/m^3) and were fed 4% of their respective biomass divided into three meals at 9 am, 12 pm, and 4 pm. The feeding ratio was adjusted after periodic biometry (every two weeks) and in case of any mortality. To adjust the feeding rate, all fish from each tank were bulk weighed by netting the fish before the first meal and transferring them to a pre-weighed container on a digital scale. The rearing system was static, and tanks were supplied with proper aeration using air stones to ensure constant oxygen supply throughout the experiment. Rearing water was exchanged daily (30%) with overnight aerated and dechlorinated water supply. The physicochemical parameters of rearing water, including pH (7.2 ±0.6), oxygen (7.5±1.14 mg/L), NH_3 (0.04±0.01 mg/L), and NO_2 (0.03±0.02 mg/L) were monitored during the experiment. The water temperature was maintained at 21±2°C under 12L:12D photoperiod where approximately 30% of the illumination was natural.

Growth performance parameters

After 60 days of dietary trial, fish were weighed, and growth-related parameters including weight gain (WG), feed conversion ratio (FCR), specific growth rate (SGR), and survival rate (SR) were measured according to the following equations:

$$WG\ (g) = final\ weight - initial\ weight$$

$$FCR = total\ feed\ given\ (g) / weight\ gain\ (g)$$

$$SGR\ (\%g/day) = [(Ln\ final\ weight\ (g) - Ln\ initial\ weight\ (g)] / days) \times 100$$

$$SR\ (\%) = (final\ number\ of\ fish / initial\ number\ of\ fish) \times 100$$

Total bacteria count and probiotic bacteria

The intestinal bacteria of three randomly selected fish from each treatment (1 fish from each replicate) were evaluated at 15, 30, 45, and 60 days of the experiment. The fish were starved for 24 h and euthanized using an overdose of clove oil (250 mg/l). The abdominal sections of the fish were cut under aseptic conditions, and the whole intestine was homogenized in saline physiology solution (0.9% NaCl). Then, the various dilutions (10^{-1}–10^{-9}/ml) were prepared and cultured on Tryptic Soy Agar (TSA) for total bacteria count. For lactic acid bacteria count, 50 µL of *E. faecium* suspension was inoculated into 20 mL of Kanamycin Aesculin Azide Agar (KAAA) (Bagheri et al., 2008).

Biochemical assay

To evaluate biochemical parameters, the blood samples were drained from 3 fish per treatment using non-heparinized 1 ml syringes at the end of the experiment. The samples were transferred to 1.5 ml tubes and left at room temperature to clot for 1 h, centrifuged (Heraeus Megafuge 16 R Centrifuge, 5000×*g*, 10 min). The serum was separated and kept at −80°C until further analysis. Biochemical parameters, including total serum protein, albumin, glucose, triglyceride, triglyceride, and cholesterol, were measured in serum using the calorimetric method (WPAS2000-UV/VIS, Cambridge, UK) via commercially available kits (Pars Azmun Co, Tehran, Iran) and analyzed by a biochemical autoanalyzer (Mindray, China). Alkaline phosphatase (ALP), alanine transaminase (ALT), and aspartate aminotransferase (AST) activities were measured using commercial kits (Pars Azmun Co., Tehran, Iran) according to the manufacturer's instructions.

Immune responses

Lysozyme activity was measured in serum according to the Ellis (1990) method. In brief, the lysozyme activity was measured by a turbidimetric method using the gram-positive bacteria *Micrococcus luteus* (Sigma, M 3770, St. Louis, MI, USA). The absorption of samples was read by a spectrophotometer (ELISA Reader) at 530 nm.

The IgM level was measured using immunoturbidimetry. A spectrophotometer measured the turbidity intensity at 450 nm. Total protein was measured using commercial kits following the manufacturer's instructions (Pars Azmoon, Tehran, Iran). Complement 3 (East BioPharm, CAT. CK-E90918) and complement 4 (East BioPharm, CAT. CK-E90919) were measured using ELISA kits (Hangzhou East BioPharm, USA) according to the manufacturer's instructions.

Statistical analysis

All statistical analyses were performed using the SPSS software package version 17. The data normality was checked using the Shapiro–Wilk test. A one-way ANOVA test was applied at the probability level of $P<0.05$ to compare the treatments, and Duncan's multiple range test was used to compare the means. All data are presented as mean ± standard deviation (SD).

Results

Growth performance

The growth parameters of fish after 60 days of feeding trial are shown in Table 2. According to the results, fish gained more weight when fed diets supplemented with *E. faecium* (EF1 and EF2) compared to both control and negative control groups (C and CP) ($P<0.05$). Similar results were found regarding FCR and SGR ($P<0.05$). A significant increase in SGR was observed in the groups supplemented with *E. faecium* (1.2±0.01% g/day for EF1 and 1.08±0.01% g/day for EF2) compared to control groups ($P<0.05$). In addition, the survival rate after 60 days was significantly higher in the EF1, EF2, and CP treatments compared to the control group (C).

Table 2. Growth performance of *Sander lucioperca* after 60 days of feeding with experimental diets, including the graded level of *Enterococcus faecium* and control diet

Treatments	C	CP	EF1	EF2
Initial weight (g)	14.4±0.3	14.5±0.4	14.4±0.3	14.5±0.5
Final weight (g)	19.5±0.7 b	20.3±0.8 b	32.5±1.3 a	29±1.2 ab
Weight gain (g)	5.1±0.05 b	5.8±0.07 c	18.03±0.1 a	15.5±0.1 a
SGR (% g/day)	00.72±0.004 b	0.78±0.01 b	1.2±0.01 a	1.08±0.01 a
FCR	1.56±0.05 a	1.43±0.05 a	0.89±0.05 b	0.83±0.07 b
SR (%)	80±0.00 b	100±0.00 a	100±0.00 a	100±0.00 a

(C) basal diet; (CP) basal diet + KAA, (EF1) basal diet + 10^{10} CFU/g of *Enterococcus faecium*; (EF2) basal diet + 10^{8} CFU/g of *E. faecium*, (FCR) feed conversion ratio, (SGR) specific growth rate, (SR) survival rate. Different letters in each row indicate significant differences (P<0.05).

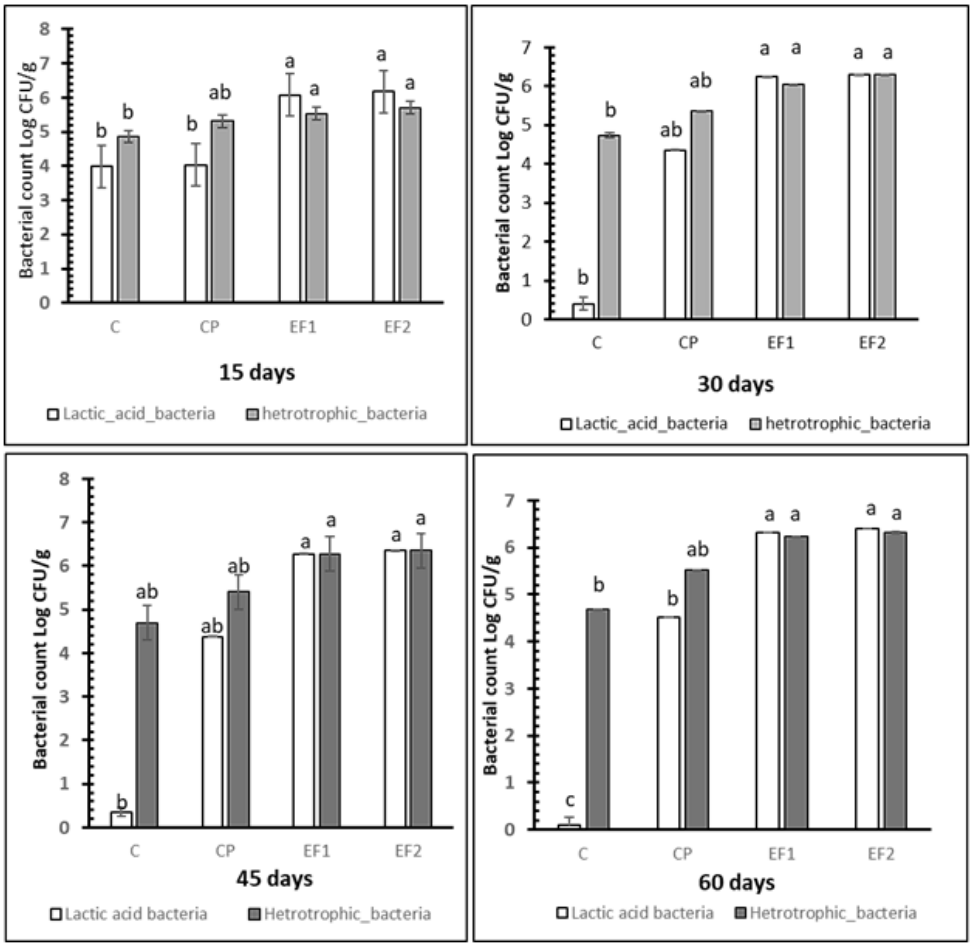

Figure 1. Bacterial count from the intestines of *Sander lucioperca* after 15, 30, 45, and 60 days of dietary trials. (C) basal diet; (CP) basal diet + KAA, (EF1) basal diet + 10^{10} CFU/g of *Enterococcus faecium*; (EF2) basal diet + 10^{8} CFU/g of *E. faecium*. Different letters indicate significant differences at P<0.05

Gut microbiota

Total bacterial counts and the average number of probiotics in the intestine of *S. lucioperca* groups fed *E. faecium* for 60 days are shown in Figure 1. After 2 weeks, the results showed that total bacterial count (LAB + heterotroph bacteria) was higher in experimental groups compared to the control group (C), where LAB was significantly increased in EF1 and EF2 (P<0.05) (Figure 1). Similar results were found at 30, 45, and 60 days of the trial. However, the number of LABs drastically decreased after 60 days in the control group (C) (P<0.05). Interestingly, at the end of the experiment, the LAB count was significantly higher in CP when compared to the C treatment (P<0.05) (Figure 1).

Table 3. Biochemical parameters of *Sander lucioperca* fed diets supplemented with *E. faecium* for 60 days

Treatment	C	CP	EF1	EF2
Glucose (mg/dL)	46.3±2.4 b	48±3.2 b	49.33±3.4 b	54.66±3.6 a
Cholesterol (mg/dL)	242.7±10.4	242±11.2	231±12.4	233±16.4
Triglycerides (mg/dL)	447.3±8.4	459.6±12.5	435.3±10.8	466.3±11.2
Total protein (g/dL)	3.1±0.3 b	3.1±0.1 b	3.6±0.07 a	3.3±0.05 ab
Albumin (g/dL)	0.95±0.04	0.97±0.05	0.963±0.1	0.956 ±0.07
AST (IU/L)	106.3±4.3 a	104.7±5.8 a	99.33±5.4 b	93.33±6.4 b
ALT (IU/L)	31.7±3.2 a	28.7±1.84 b	29±5.4 b	28.33±3.2 b
ALP (IU/L)	167.7±12.4 a	158.3±15.2 b	160.33±18.2 ab	155.33±14.8 b

(C) Basal diet; (CP) basal diet + KAA, (EF1) basal diet + 10^{10} CFU/g of *Enterococcus faecium*; (EF2) basal diet + 10^{8} CFU/g of *E. faecium*. Different letters in each row indicate significant differences between the experimental groups (P<0.05).

Table 4. Immunological parameters of *Sander lucioperca* fed diets supplemented with *E. faecium* for 60 days

Treatment	C	CP	EF1	EF2
Lysozyme (U/ml)	23±1.33 b	23±1.66 b	28±1.33 a	26±2.3 a
IgM (mg/dl)	20±2 b	20±3.2 b	25.33±2 a	22.66±3 ab
C3 (mg/dl)	25.33±3.4 b	27.33±5.8 b	30.33±4.2 a	26.66±2.8 b
C4 (mg/dl)	28.3±2.6 b	29.33±4.2 b	32.33±3.4 a	30.66±2.6 ab

(C) Basal diet; (CP) basal diet + KAA, (EF1) basal diet + 10^{10} CFU/g of *Enterococcus faecium*; (EF2) basal diet + 10^{8} CFU/g of *E. faecium*. Different letters in each row indicate significant differences between the experimental groups (P<0.05).

Biochemical parameters

Table 3 represents the serum biochemical parameters of fish fed 60 days with different diets supplemented with *E. faecium*. The glucose level in EF2 (10^{8} CFU/g) was higher than other treatments (P<0.05), while a lower level of cholesterol, however not significant, was found in the group fed with probiotics (P>0.05). As for triglyceride, higher levels were found in EF2 and CP treatments compared to other groups (P>0.05). The total protein level of individuals from EF1 treatment was significantly higher than other treatments (P<0.05). The albumin level was not affected by different dietary treatments.

As for plasma enzymes, AST was lower in EF2 treatment than in C and CP treatments; however, it was not statistically different from EF1. For ALT, all treatments showed lower values compared to control (C) (P<0.05), while ALP levels were the lowest in EF2 followed by CP treatment; both were significantly different from other treatments (P<0.05).

Immunological parameters

Serum immunological responses of fish after 60 days are represented in Table 4. Results showed that all measured parameters were significantly higher in fish from EF1 treatment compared to control groups (P<0.05), while only lysozyme activity was statistically different from control groups. However, no significant differences were found between EF1 and EF2 for all measured parameters except C3 activity. In addition, no significant differences were found between the C and CP groups for all measured parameters (P>0.05).

Discussion

Probiotics have shown significant success in aquaculture, fulfilling various functions, including enhancing growth, boosting immunity, and increasing disease resistance in target species. The present results indicated that the growth parameters of *S. lucioperca*, including SGR, WG, FCR, and SR, were significantly enhanced when diets were supplemented with 10^{8} and 10^{10} CFU/g *E. faecium*. Da Costa Sousa et al. (2019) showed that *E. faecium* regulates the intestinal microbiota and improves the growth of *Arapaima gigas*. The growth rate and weight gain of *Arapaima gigas* fed diets containing *E. faecium* increased by 8.6% and 39.24%, respectively, compared to those without *E. faecium*. Similarly, 10^{8} CFU/g *Lactobacillus plantarum* was found optimal for enhancing the growth performance of *Labeo rohito* (Giri et al., 2014). In the present study, there was a significant difference between the survival rates of the control groups (C and CP), possibly due to a higher level of lactic acid bacteria in the CP control group. It is suggested that probiotics increase fish survival by stimulating an intestinal immune response (Aly et al., 2008; El-Saadony et al., 2021; Mirbakhsh et al., 2023; Zorriehzahra et al., 2016), thus enhancing the survival rate of fish.

E. faecium, a member of LABs, is commonly used as a probiotic in fish farming and is known to be present in the intestines of healthy fish. LAB in intestinal tracts of fish is reported to exert a wide range of biological activities, including antibacterial activity by producing bacteriocins (Sonsa-Ard et al., 2015) and helping metabolism

by excreting enzymes that facilitate the digestion of food, reducing the intestinal pH and consequently increasing the absorption of minerals (Sun et al., 2012). *E. faecium* isolates from other species have been tested in fish. For instance, Wang et al. (2008) found that 10^7 CFU/ml inoculation of a strain of *E. faecium* (*E. faecium* ZJ4) isolated from piglets into the rearing water increases the growth of Nile tilapia (*Orechromis niloticus*). Our study revealed that a continued supply of *E. faecium* helped dominate the intestine, which could be the reason for improved fish growth performance. It is important to note that in our study, the number of heterotrophic bacteria also increased, which requires further microbiome analysis research to understand the dynamics of the populations. In addition, histological evaluations were performed on intestinal samples, indicating improvement in general indicators of healthy fish intestines (unpublished results).

Bogut et al. (1998) reported that *E. faecium* in fish can adhere to intestinal epithelial cells. Our results showed the ability of dietary *E. faecium* to colonize and grow in the intestine of *S. lucioperca*. In addition, diet supplementation with KAA medium changed the microbiome, favoring LAB in CP treatment. Furthermore, the results showed that the heterotrophic bacteria were slightly higher in all experimental groups compared to the control group (C), although results were only statistically different after 2 weeks. Tachibana et al. (2020) have reported that *E. faecium* can enhance the growth performance of Nile tilapia and suggested the efficient 2-week periodic application. Furthermore, Bagheri et al. (2008) suggested the increase of probiotic bacteria in the intestine owing to the continual application of probiotics. Our results were similar to previous findings from studies on rainbow trout (*Oncorhynchus mykiss*) (Balcázar et al., 2006; Nikiforov-Nikishin et al., 2022), African catfish (*Clarias gariepinus*) (Al-Dohail et al., 2009), *S. lucioperca* (Faeed et al., 2016), and *Labeo rohita* (Giri et al., 2014).

Based on the present results, the immune status of fish was enhanced. Lysozyme is one of the most important components of non-specific immunity. The increase in serum lysozyme activity indicates the improvement of the immune status of the fish immune system against infectious agents and stress (Saurabh and Sahoo, 2008). Moreover, the total Ig levels of fish were heightened in probiotic-receiving groups. Another important part of fish's innate immune system is the complement system, whose activation contributes significantly to the orchestration and development of an acquired immune response (Boshra et al., 2006; Harikrishnan et al., 2021). In this study, we observed increased activity of both C3 and C4 complement components in response to probiotic supplementation. *E. faecium* in tilapia (Wang et al., 2008), *E. gallinarum* in marine aquaculture species (Román et al., 2015) and *E. casseliflavus* in rainbow trout (Safari et al., 2016), *Pediococcus acidilactici* in common carp (*Cyprinus carpio*) (Maniat et al., 2023) have led to the improvement of immune indicators such as serum lysozyme activity, C3, and C4. The enhancement of immune responses could be partially due to the immune system's interaction with the changes in gut microbiota composition or induced immunity caused by secreted metabolites (e.g. antimicrobials and vitamins) (Ohira et al., 2017; Salari et al., 2021).

Cholesterol is important in cell membranes' structure and biosynthesis of some hormones (Tocher, 2003). Part of cholesterol is provided through food and part through synthesis in the liver. In this research, cholesterol and triglycerides in probiotic treatments were lower than in control treatments; however, there were no significant differences. Bajelan et al. (2018) reported that using symbiotics in fish diet led to a decrease in low-density cholesterol and triglycerides compared to the control group, which was consistent with the present study. It has been suggested that probiotics help promote the β-oxidation process and intestinal absorption of fatty acid metabolites (Nguyen et al., 2018). Lower cholesterol levels could also be attributed to fish's general well-being and health status. In addition, present results suggest a potential involvement of *E. faecium* in lipid metabolism, which merits further investigation.

The complex interaction between the gut-associated lymphoid tissue (GALT) and microbiota is not clearly understood. Several studies have reported that the total protein level increases when probiotics are used (Imanpoor and Roohi, 2015). In this research, total protein in probiotic treatments significantly increased, especially *E. faecium* 10^{10} CFU/g. This could be attributed to the changes in gut microbiota composition, which may result in a systemic response. The increase in the amount of total protein in the blood plasma can be due to the strong non-specific responses of fish caused by the effect of probiotic bacteria or because of increasing the ability to deal with disease, infection, and acute or chronic inflammation (Imanpoor and Roohi, 2015).

We also observed that 10^8 CFU/g *E. faecium* in the diet caused a significant increase in glucose compared to other treatments. This contradicts some reports (Tachibana et al., 2020) and complies with others (Gayed et al., 2021). We did not measure cortisol in the current experiment. However, it is known that the type of probiotic, concentration (CFU/g feed), and incorporation methods can cause variable results (Mohammadian et al., 2022). In this research, it was observed that the activity level of serum liver enzymes (AST, ALT, and ALP) in fish that received probiotics, especially the treatment of *E. faecium* 10^8 CFU/g diet, caused a significant increase in glucose compared to other treatments. In addition, the type of probiotic, concentration (CFU/g feed), and incorporation method can probably cause these differences (Mohammadian et al., 2022).

In conclusion, autochthonous *E. faecium* can be considered a promoter of the intestinal microbiota in pikeperch and could increase the production yield of pikeperch aquaculture. Based on the results, it is suggested that a 10^{10} CFU/g diet of *E. faecium* can be used to enhance the growth and immunity of *S. lucioperca.* It is also

recommended that future studies consider the interaction between microbiota and gut-associated lymphoid tissue.

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