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*Research Article*

# Autochthonous probiotic in Asian sea bass *(Lates calcarifer)* diet: reduces excessive liver lipid deposition and resistance against *Streptococcus iniae* infection

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**Abstract**

Aquaculture represents a pivotal economic sector worldwide, meeting the escalating food demands of the expanding global population. Consequently, this research aimed to assess the incidence of fatty liver in Asian sea bass (*Lates calcarifer*) subjected to a diet enriched with lactic acid bacteria and evaluate their survival against *Streptococcus iniae* infection. The present study examined 240 sea bass (109 ± 10.5 g average weight) that were randomly assigned into four treatments with three replicates (25 specimens per treatment) for 60 days. The treatments comprised the following: First treatment: fish were fed with commercial feed. Second treatment: fish were provided with feed containing 109 CFU/g of *Lactobacillus plantarum* bacteria. Third treatment: fish were fed with feed containing 109 CFU/g of *Lactobacillus pentosus* bacteria. Fourth treatment: fish were provided with feed having 109 CFU/g of *L. pentosus* bacteria combined with *L. plantarum* in equal proportions. At the end of the experiment, the growth performance, the survival rate against the pathogenic bacteria *S. iniae* and the amount of fatty liver were evaluated. The findings disclosed enhanced growth indicators in the second treatment (strain 140) during the initial 30 days. Furthermore, statistically significant disparities were noted in the third treatment (2P) concerning PER, SGR, WG, RGR, and DWG during the subsequent 30-day period ($P<0.05$). Liver pathology examination demonstrated that most treatments resulted in the development of fatty liver. However, the third treatment (*L. pentosus*) exhibited the lowest incidence of fatty liver when endogenous probiotics were incorporated into the diet. Post-challenge with *S. iniae*, the mortality rate in the probiotic treatments *L. pentosus* (P2) and *L. plantarum* (140) significantly surpassed that of the control group ($P<0.05$). The findings underscore the absence of synergistic interactions between the two experimental probiotics on the 60th day, as the combined group displayed diminished growth performance compared to the individual groups. Moreover, the use of *L. plantarum* and *L. pentosus* bacteria, particularly the latter, has been shown to significantly improve several growth indicators, as well as the food conversion ratio. Consequently, these probiotics are recommended as dietary supplements for Asian sea bass.

## Introduction

Given the prevailing challenges, such as climate change, freshwater scarcity and an increasing demand for nutritious food, which is growing in line with population growth, there is a strategic imperative for governments and non-governmental organizations involved in aquaculture to adopt policies that encourage fish farming and the cultivation of other aquatic organisms in sea-water environments (Ahangarzadeh *et al*., 2023). This imperative is particularly pertinent to nations with extensive coastal domains, such as Iran, which, boasting a coastal strip spanning 5800 km, is endowed with a promising capacity for harnessing suitable water resources for the culture of marine aquatic species. Nevertheless, the development of marine aquaculture is constrained by a number of challenges, particularly those related to species diversity and the availability of preferred species that can rapidly proliferate and are adaptable to local climatic and meteorological conditions. One solution that is emerging in response to these challenges is the use of brackish and marine waters for aquaculture. This solution offers a pragmatic approach to addressing the complex interrelationship between climate changes, freshwater scarcity and growing food requirements. Iran's expansive coastal strip offers considerable potential for the sustainable development of marine aquaculture, but the industry must overcome challenges related to species diversity, adaptation to local conditions, and market alignment if it is to remain viable. In order to achieve this, it is necessary to establish collaborative relationships between governmental and non-governmental organizations. These partnerships will enable the identification and resolution of the obstacles currently impeding the growth of marine aquaculture, while simultaneously ensuring the environmental and economic sustainability of this sector. One illustrative example is the Asian sea bass, also known as barramundi. This fish is of significant economic importance in Southeast Asia and is widely cultivated in Australia, Thailand, and Indonesia. Its carnivorous nature and voracious behavior make it a commercially significant species in the aquaculture industry. The necessity of high-protein artificial feed in the cultivation environment emphasizes the necessity for the implementation of sustainable aquaculture practices to ensure economic viability while minimizing environmental impacts. Achieving a balance between the nutritional requirements of sea bass and environmental considerations is of pivotal importance to guarantee the enduring success of barramundi production. Feed formulation constitutes a significant portion, up to 70%, of the total production expenses in the aquaculture sector. The optimization of metabolic nutrient absorption represents a pivotal objective in contemporary aquaculture production. By reducing feed costs, this objective has the potential to enhance overall aquaculture profitability, thereby contributing to the viability and sustainability of the aquaculture sector (Ibrahem *et al*., 2010). The intestinal microbiota plays a pivotal role in regulating host nutrient digestion, absorption, and metabolism. Previous studies have demonstrated the intricate interplay between gut microbial functional metabolites and lipid metabolism. The host's lipid metabolism is affected by a number of key factors, including short-chain fatty acids (SCFAs), bile acids, lipopolysaccharides, trimethylamines, tryptophan and their derivatives. Understanding these relationships sheds light on potential avenues for modulating lipid metabolism in host-microbiota interactions. In recent years, there has been growing interest in the use of endogenous probiotics as dietary supplements to improve growth rate, immunity, and disease resistance in fish (Kokou *et al*., 2019; Wang *et al*., 2018; Xia *et al*., 2020). Crucially, the probiotic must be species-specific to ensure compatibility with the target species. This compatibility enhances the probiotic's ability to compete with native intestinal microbes and establish itself in the new host environment. The antimicrobial effects of probiotics are diverse, encompassing the production of antibiotics, bacteriocins, siderophores, lysozymes, proteases, and the induction of pH changes through the production of organic acids (Sugita *et al*., 1996). A complex area of research is the intricate interplay between probiotics, gut microbiota, and host metabolism. While evidence supports the impact of gut microbiota on metabolism, the definitive efficacy of using probiotics alone or in combination remains uncertain. Continued research is critical to understanding the roles and potential benefits, particularly given the physical separation between the gut and the liver, which is the critical organ that regulates metabolic activity, hormone production, detoxification, and immune response (Du, 2014). In fish farming, liver diseases present a significant challenge, often attributed to imbalanced commercial diets not tailored to the target species (Du, 2014; Mohtashamipour *et al*., 2023). Disruptions in food metabolism can lead to metabolic disorders, culminating in liver damage (Francis *et al*., 2001). The fermentation activity of endogenous probiotics produces metabolites like acetate, propionate, and butyrate, with all short-chain fatty acids (SCFAs) playing crucial roles in host metabolism. These SCFAs serve as energy substrates in lipogenesis, gluconeogenesis, and cholesterol synthesis, acting as signaling molecules in fish lipid metabolism (Besten *et al*., 2013, 2015). Acetate, the primary SCFA in animals, regulates lipid metabolism by inhibiting fat cell differentiation and reducing fat storage. As highlighted earlier, intestinal microbiota and endogenous probiotics significantly regulate host metabolism, influencing both overall host and distant liver metabolism (Benakis *et al*., 2020; Ezra-Nevo *et al*., 2020). Using probiotics as a recommended approach to mitigate issues associated with artificial diets. Incorporating probiotic food supplements into the diet has demonstrated the potential to reduce fat. Current research focuses on integrating endogenous probiotics into the diet to enhance the liver's tissue structure and overall health.

## Materials and methods

### *Bacterial strains*

In this research, *Lactobacillus pentosus* and *Lactobacillus plantarum* bacteria, which were isolated from a number of Marine fish such as *Scomberomorus* *guttatus*, *Lutjanus malabaricus*, *L. calcarifer*, etc, as probiotics with good performance, were used to evaluate the best growth performance at the cultural temperature of Asian sea bass fish (Our previous study).

*Microbiological tests*

To verify the tested Lactobacillus bacteria, the frozen cultures of the above-mentioned bacteria were recovered on, MRS broth, and incubated anaerobically for 24-48 hours. Subsequently, a complete loop of the grown bacteria was cultured in MRS agar under the same conditions. After 24-48 hours, with confirmed bacterial purity, a gram staining procedure was conducted, and their morphology was observed using a light microscope. For confirming *Streptococcus inaie* bacteria, they were cultured in TSB at 37°C for 24-48 hours A complete loop of each culture was inoculated into TSA and incubated at 37°C for 24-48 hours. After ensuring the samples' purity, Gram staining, catalase, and oxidase tests, and a sugar fermentation test (as previously described) were performed (Suneel and Basappa, 2013). Following the phenotypic and biochemical test of Lactobacillus bacteria, S RNA16 ribosomal nucleotide sequencing was employed for confirmation. Bacterial DNA extraction was performed using a kit (Sina Clone, Iran). Subsequently, the obtained sequencing results were verified using BioEdit software, and the nucleotide sequence was compared with available sequences on the relevant site. After receiving the sequencing results, the relevant sequence was checked by BioEdit software. Finally, the nucleotide sequence of the obtained sequences was compared with the nucleotide sequence available in the NCBI gene bank (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

*Experimental designed*

In this study, 300 Asian sea bass juveniles, (109±10.5 g), were obtained from the Ramoz breeding center and transferred to the Faculty of Veterinary Medicine at the Shahid Chamran University of Ahvaz. Following an adaptation period, the juveniles were randomly divided into four treatments with three replicates, each consisting of 25 juveniles. The duration of the research was 60 days, excluding a two-week adaptation phase and before the challenge test. Five air stones connected to a central aerator were placed in each tank to ensure optimal oxygen levels for the fish. The research facility maintained a light cycle of 10-12 hours of light and 14-12 hours of darkness. Water quality parameters, including dissolved oxygen (8.7±1.3 mg/L), temperature (29.1±1.5°C) pH (7.94±0.11) and total ammonia nitrogen (< 0.01 mg/L) were measured. The experimental treatments are as follows: First, fed with commercial feed. The second treatment was fed with $10^9$ CFU/g of *Lactobacillus plantarum* bacteria in the diet. The third treatment was fed with $10^9$ CFU/g of *L. pentosus* bacteria in the diet. Third treatment: fed with $10^9$ CFU/g *L. pentosus* combined with *L. plantarum* in equal proportion in the diet.

*Diet preparation*

By the methods recommended by Planas *et al*. (2004) and Vine *et al*. (2004), the preparation of probiotic bacteria and their inoculation into fish food followed a specific protocol. Each bacterium was cultivated separately in MRS broth under anaerobic conditions. Following growth, the bacteria were precipitated and washed through centrifugation (3000 revolutions for 5 min), and their concentration in physiological serum was adjusted to $3\times10^9$ CFU/ml using standard McFarland tubes. Subsequently, a suspension containing *Lactobacillus plantarum* and *Lactobacillus pentosus* bacteria was added to 100 g of fish food. Each probiotic was then sprayed onto the food with a $1\times10^9$ CFU/mL concentration/g food. Sampling from the prepared foods was performed to verify food's bacterial count was determined. For the control group, the food was sprayed only with sterile normal salin (Planas *et al*., 2004; Vine *et al*., 2004; Mohammadian *et al*., 2016, 2017). This meticulous procedure aimed to ensure the precise incorporation of probiotic bacteria into the fish food for subsequent feeding trials.

*Growth performance*

To determine the growth performance, all fish within each treatment were individually weighted at the beginning and sixth week of the trial. The weight of all fish in each tank was determined every two weeks, and feed ratios were adjusted according to the fish weight. Growth parameters including Relative growth rate (RGR), weight gain (WG), specific growth ratio (SGR), condition factor (CF), feed conversion ratio (FCR), and protein efficiency ratio (PER) were calculated for each group as follow:

DWG = (WF – WI) / days

RGR = [Δw (g) / IBW (g)] × 100

SGR (% body weight / days) = [(Ln $W_F$ − Ln $W_I$) / t] × 100

CF = (FW × 100) / standard length3 (cm)

FCR = feed intake (g) / weight gain (g)

PER = protein intake (g)⁄weight gain (g)

Where, $W_I$ is initial body weights; $W_F$ is final body weights (g); and t is the trial duration in days

*Sampling*

Liver samples were obtained on days 60 from the beginning of the experiment, with nine fish randomly selected from each treatment group. Prior to sampling, all fish underwent a 24-hour fasting period. Anesthesia was induced using 100 mg/L of Clove oil, per the methodology established by Coyle *et al*. (2004). Whole tissue specimens were collected from the fish, according to Hoseinpouri Ghasemabad Sofla *et al*. (2024).

*Liver histopathology analysis*

Paraffin sections of the liver were stained with oil-red O and hematoxylin–eosin by Wuhan Google Biological Technology Co., Ltd. (Wuhan, China). Under light microscopy, three fields were randomly observed for each sample. All images were marked and analyzed by Image-Pro Plus 6.0.

*LD*50 *and challenge test*

Before starting the main study, the lethality of *Streptococcus iniae* in Asian seabass was evaluated by the following method. *S. iniae* was cultured in a TSB for 48 hours at 37°C. Subsequently, the culture medium centrifugation at 3500 rpm for 10 minutes, and was twice washed with normal saline. An adequate amount of normal saline was added to the bacteria to achieve turbidity equivalent to McFarland tube number 10 (CFU/mL $3\times10^9$). The resulting suspension was then diluted to obtain suspensions ranging from $10^5\times1$ to $10^9\times1$. For each dilution of bacteria, six Asian sea bass weighing approximately 100 g were injected intraperitoneally. A control group was injected with sterile normal saline. Fish mortalities were recorded over ten days, and post-mortem confirmation of bacterial infection (*S. iniae*) was attained by culturing internal organs. Utilizing Probit software, the LD50 was calculated as $10^7$ (Halimi *et al*., 2018), where pathogens represent the absorbance values at 620 nm wavelength for the bacterial suspension strain. Following the determination of the LD50 for *S. iniae* bacteria in Asian sea bass, after the 60-day feeding period with research probiotics, half of the fish in each replication (10 specimens) were intraperitoneally challenged with a dose causing a 50% mortality rate after 14 days of exposure to *S. iniae* bacteria. Subsequently, losses were investigated across different treatments. This approach aimed to evaluate the impact of the probiotics on fish survival post-pathogenic challenge.

**Results**

*Growth performance*

In summary, on the 30th day, the second treatment (*Lactobacillus plantarum*) exhibited the highest weight gain, specific growth factor, and protein efficiency, with significant differences from the control and third treatments. The fourth treatment showed the lowest food conversion ratio. On the 60th day, the third treatment had the highest weight gain, specific growth coefficient, and nutritional efficiency, not significantly different from the second treatment. The fourth treatment consistently showed lower values across various parameters. Overall, treatments with *Lactobacillus plantarum* demonstrated favorable outcomes in multiple growth and efficiency measures (Table 1).

*Histopathology of liver*

In summary, a semi-quantitative pathology study assessed fatty liver damage in fish. Microscopic slides from each fish's liver were examined by scoring +1 for microvesicles less than 25%, +2 for 25-75%, and +3 for over 75%. On the 60th day, the third treatment (*Lactobacillus pentosus*) showed the lowest fatty liver level, significantly different from other treatments ($p$<0.05). Grade two microscopic slides predominated in this treatment, indicating a higher percentage of normal hepatocytes. Conversely, the control treatment exhibited the most severe fatty liver damage, with grade three slides showing extensive fat vacuoles and compressed nuclei. Various degrees of fatty liver were observed in other treatments (Figs. 1 and 2).

*Bacterial challenge*

As depicted in the graph, the treatments *Lactobacillus pentosus*, *Lactobacillus plantarum*, and the mixed treatment (fourth) exhibited the slightest losses, respectively, demonstrating a significant difference compared to the control group ($p$>0.05).

**Table 1: Growth performance of Asian seabass fed either regular feed or feed supplemented with probiotics for 60 days**

| Parameters | Groups | Day 30 | Day 60 |
|---|---|---|---|
| IW | Control | $113.2\pm4^{a}$ | $189.8\pm7^{b}$ |
| | *Lactobacillus plantaum* | $111.3\pm0.11^{a}$ | $194.6\pm0.52^{a}$ |
| | *L. pentosus* | $112.6\pm0.52^{a}$ | $189.53\pm0.5^{b}$ |
| | Mixed | $112.6\pm0.52^{a}$ | $191.53\pm0.5^{ab}$ |
| FW | Control | $210\pm8^{b}$ | $270\pm8.7^{b}$ |
| | *L. plantaum* | $222.12\pm0.82^{a}$ | $291.34\pm0.56^{a}$ |
| | *L. pentosus* | $215.48\pm0.5^{ab}$ | $294.36\pm0.55^{a}$ |
| | mixed | $218.24\pm0.67^{ab}$ | $255.93\pm0.9^{b}$ |
| WG% | Control | $76.6\pm3^{b,A}$ | $60\pm0.7^{b,B}$ |
| | *L. plantaum* | $83.4\pm0.61^{a,A}$ | $69.22\pm0.35^{b,B}$ |
| | *L. pentosus* | $76.93\pm1^{b,A}$ | $78.88\pm1.02^{a,A}$ |
| | mixed | $78.93\pm0.11^{b,A}$ | $37.69\pm1.56^{c,B}$ |
| FCR | Control | $1.53\pm0.06^{b,A}$ | $1.5\pm0.02^{b,A}$ |
| | *L. plantaum* | $1.53\pm0.01^{b,A}$ | $1.2\pm0.006^{a,B}$ |
| | *L. pentosus* | $1.61\pm0.02^{b,A}$ | $1.08\pm0.01^{a,B}$ |
| | mixed | $1.48\pm0.002^{a,A}$ | $1.38\pm0.05^{ab,A}$ |
| SGR | Control | $1.47\pm0.004^{b,A}$ | $0.71\pm0.01^{b,B}$ |
| | *L. plantaum* | $1.6\pm0.009^{a},^{A}$ | $0.77\pm0.005^{b,B}$ |
| | *L. pentosus* | $1.48\pm0.02^{b,A}$ | $0.89\pm0.01^{a,B}$ |
| | mixed | $1.51\pm0.006^{b,A}$ | $0.45\pm0.01^{c,B}$ |
| PER | Control | $1.48\pm0.06^{b,A}$ | $1.51\pm0.02^{b,A}$ |
| | *L. plantaum* | $2.13\pm0.01^{a,A}$ | $1.56\pm0.008^{b,B}$ |
| | *L. pentosus* | $1.96\pm0.02^{a,A}$ | $1.77\pm0.02^{a,A}$ |
| | mixed | $1.95\pm0.002^{a,A}$ | $0.823\pm0.334^{c,B}$ |
| DWG | Control | $2.1\pm0.08^{a,A}$ | $1.71\pm0.02^{b,A}$ |
| | *L. plantaum* | $2.38\pm0.017^{a,A}$ | $1.97\pm0.01^{ab,B}$ |
| | *L. pentosus* | $2.19\pm0.02^{a,A}$ | $2.25\pm0.02^{a,A}$ |
| | mixed | $2.25\pm0.003^{a,A}$ | $1.076\pm0.044^{c,B}$ |
| RGR | Control | $67.6\pm0.25^{a,A}$ | $28.5\pm0.75^{ab,B}$ |
| | *L. plantaum* | $75.15\pm0.61^{a,A}$ | $31.16\pm0.26^{a,B}$ |
| | *L. pentosus* | $68.3\pm1.21^{a,A}$ | $36.6\pm0.55^{a,B}$ |
| | mixed | $70.1\pm0.37^{a,A}$ | $17.27\pm0.76^{b,B}$ |
| FER | Control | $65.3\pm2.7^{a,A}$ | $66.6\pm1.2^{b,A}$ |
| | *L. plantaum* | $65.33\pm0.47^{a,B}$ | $83.2\pm0.44^{a,A}$ |
| | *L. pentosus* | $61.7\pm0.8^{a,B}$ | $91.76\pm1.18^{a,A}$ |
| | mixed | $67.12\pm0.09^{a,A}$ | $72.08\pm3.02^{b,A}$ |

***** For each parameter, values (Mean ± SD) bearing different lowercase letters or different uppercase letters represent significant differences within each column or each row, respectively ($p$<0.05).

* Abbreviations: IW, Initial Weight; FW, Final Weight; %WG, Weight Gain%; FCR, Feed Conversion Ratio; SGR: Specific Growth Rate; DWG, Daily Weight Gain; PER, Protein Efficiency Ratio, RGR, Relative Growth Rate; FER, Feed Efficiency Ratio.

Moreover, fourth (combined) treatments, respectively, had a significant difference compared to the control group ($p$>0.05) (Table 2).

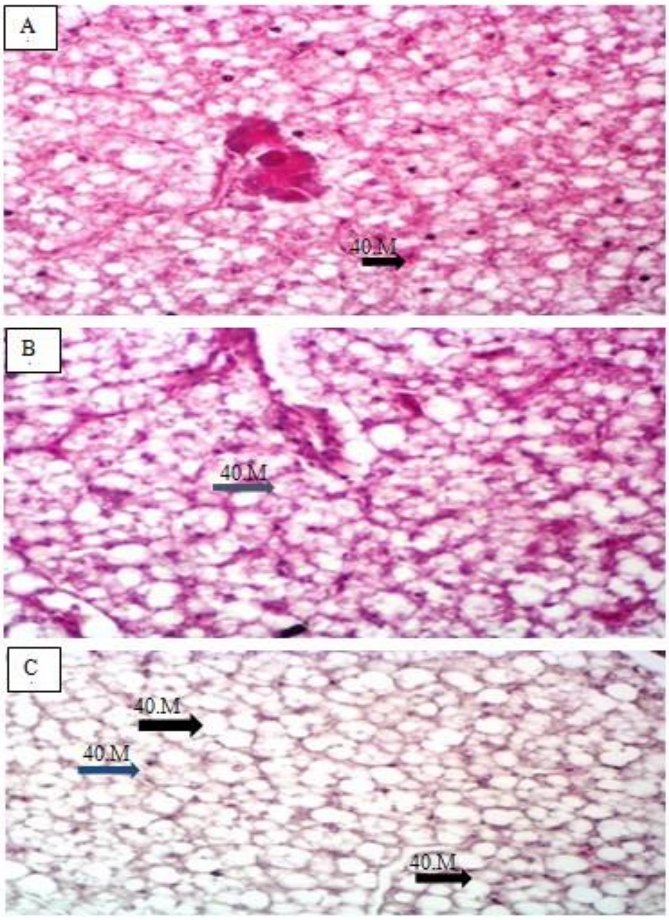


**Figure 1: Histopathologic view of liver tissue sections (40× magnification). Note the presence of macro vesicles (blue arrow) and microvesicles (black arrow) in liver hepatocytes (arrow). A), Grade No. 1; The nuclei in most of the hepatocytes have kept their central position, and fat vacuoles were observed in a smaller number of hepatocytes. B) grade number 2: In some places, the nuclei have kept their central position, and fat vacuoles were observed in a moderate number of hepatocytes. C) grade number 3: Many nuclei have lost their central position and have been pushed to the margins, and fat vacuoles were observed in many hepatocytes.**

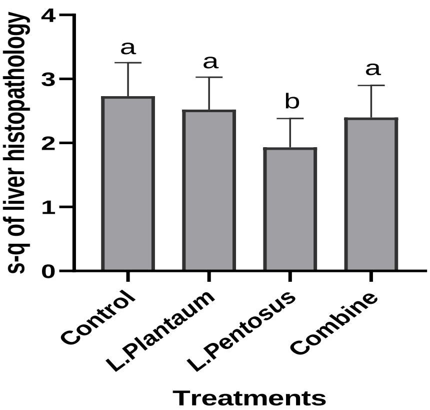


**Figure 2: Results of semi-quantitative examination of liver histopathology in different treatments (results are reported based on SD Means). * The numbers 1-4 correspond to the different degrees in the semi-quantitative survey. * Non-synonymous Latin lowercase letters on the standard deviation indicate a significant difference at 0.05 in each column.**

**Table 2: Mortality rate percentage in different treatments after challenge with *Streptococcus iniae*.**

| Parameters | Treatments | | | |
|---|---|---|---|---|
| | Control | *Lactobacillus plantaum* | *L. pentosus* | Combine |
| Mortality rate (%) | 40.6[b] | 20.3[a] | 16.63[a] | 26.6[a] |

The data represent the Mean±SD of three tanks per treatment. Values with various lowercase letters in each row indicate significant differences ($p<0.05$).

## Discussion

Using indigenous probiotics in aquaculture, especially those isolated from aquatic animals can offer advantages. These probiotics, often comprising lactic acid bacteria, are more closely aligned with the host's microbial community, potentially enhancing their effectiveness in supporting the host's health and preventing competition from other bacteria in the aquaculture environment. This tailored approach may contribute to better adaptation and overall success in maintaining a healthy microbial balance in aquaculture systems. The study highlights the efficacy of *Lactobacillus plantarum* (second treatment) and the combined treatment in significantly improving growth indicators during the initial 30 days, including specific growth factors, food conversion factors, and efficiency factors. Although there was a decreasing trend in the second 30 days, *Lactobacillus pentosus* (third treatment) and *L. plantarum* still showed improvement in growth factors. Over the entire 60-day period, all groups experienced a decrease in growth indicators compared to the first 30 days, except for an improvement in the food conversion index. Utilizing native bacterial species and lactic acid isolates from aquatic animals' digestive systems appears crucial, offering advantages such as preserving native microbial and genetic diversity, compatibility with native microbiota, and economic efficiency in probiotic extraction. The study underscores the significance of utilizing local isolates of lactic acid bacteria from the digestive systems of aquatic animals as probiotic sources in aquaculture, anticipating enhanced productivity and health. Despite the expectation of improved performance through a combination of probiotics to diversify intestinal microbiota, the results indicate a lack of synergistic effects on day 60. Surprisingly, the growth performance in the combined group was lower than in the individual groups, suggesting a potential reduction in intestinal digestibility and apparent digestibility of total nutrients due to increased competition for feed-provided nutrients by the probiotic bacteria in the combined group. This finding emphasizes the complexity of interactions among probiotic strains and highlights the need for careful consideration in designing probiotic combinations for optimal outcomes in aquaculture (Salam *et al*., 2021). Indeed, the effectiveness of probiotics in improving growth performance can vary, as highlighted by different studies. Liu *et al*. (2020) and Xia *et al*. (2020) reported favorable outcomes with a combination of fish-isolated probiotics. However, it is important to acknowledge different results, such as studies by Cerezuela *et al*. (2012, 2013) showing intestinal damage in sea bream (*Sparus aurata* L.) with probiotic supplementation, and the study conducted by Sun *et al*. (2011) did not demonstrate a significant advantage for growth and survival in groupers fed diets containing Bacillus strains. The selection and establishment of strains in the digestive system play a pivotal role in influencing the concentration of probiotics in the digestive system. This variability might explain the contrasting results in different studies and underscores the importance of considering endogenous probiotics. Research in this field indicates that there is considerable diversity among these microorganisms and that their effects on their hosts can be diverse. This highlights the importance of precision and further exploration of the use of probiotics in aquaculture industries.

Probiotics can be effective in preventing and reducing fatty liver in fish. Research has shown that probiotics can improve the condition of fatty liver and related factors such as increased fat accumulation in the liver and liver inflammation. Probiotics help improve digestion and facilitate nutrient absorption by balancing the microbial balance in the gut. The efficacy of probiotics in reducing fat accumulation in the liver and alleviating liver inflammation is contingent upon factors such as the type, proportion, and dosage of microorganisms used. The study demonstrates that *L. pentosus* treatment on the 60th day significantly reduced fatty liver compared to other experimental treatments, likely through improved digestion, absorption, and enhanced hepatocyte cell function. This endogenous probiotic appears to positively influence various liver functions, including fat metabolism, absorption of vitamins and minerals, and the reduction of liver glycogen and ALP enzyme, leading to a notable difference on the 60th day. Control treatments exhibited the highest fatty liver content. Consistent with these findings, Ruiz *et al*. (2020) reported improved liver function and enhanced liver repair in Nile tilapia fish with *Lactobacillus plantarum*. In European bass, probiotic yeast supplementation improved liver morphology and mitigated steatosis with fatty degeneration (Panagiotidou *et al*., 2016). These results underscore the potential of specific probiotics in addressing fatty liver issues in fish, emphasizing the importance of carefully selecting and applying probiotic strains in aquaculture practices. Research by Geurden *et al.* (2014) on rainbow trout and Zhou *et al*. (2021) on largemouth bass highlights that a high-carbohydrate diet can disrupt gut microbial balance: When metabolized by intestinal microorganisms, carbohydrates produce acetate, propionate, butyrate, and lactate. Acetate, in particular, regulates hepatic fat metabolism, facilitates bile acid excretion, inhibits hepatic lipid synthesis, and reduces cholesterol and triglycerides. Activation of the AMPK signaling pathway by acetate influences lipid oxidative breakdown and synthesis in the liver (Li *et al*., 2018). Studies on European sea bass by Castro *et al*. (2015) and Chinese perch by Feng *et al*. have shown contrasting effects of high-carbohydrate diets on liver fat deposition. In the present study on Asian sea bass, investigating the effects of endogenous probiotic dietary supplements revealed potential benefits. These supplements induce acetate production in the fish intestine, which may be transferred to the plasma. Acetate activation of AMPK in the liver leads to significant changes in the expression of genes related to fat metabolism, enhancing the oxidative breakdown of fat and reducing fat deposition in the liver. This underscores the potential of endogenous probiotics to modulate fat metabolism in aquaculture. The study demonstrates that introducing *S. iniae* as a bacterial challenge after 60 days revealed the protective effects of *L. pentosus* and *Lactobacillus plantarum* in Asian sea bass. The control treatment without Lactobacillus bacteria showed a mortality rate of 40.6%, while treatments with *L. pentosus* and *L.plantarum* exhibited significantly lower death rates of 16.63% and 20.3%, respectively. These findings suggest that these Lactobacillus bacteria enhanced fish immunity and resistance against the pathogenic agent *S. iniae*. Notably, *L. pentosus*, which also contributed to higher growth performance, likely induced stronger immune stimulation than other isolates, resulting in increased survival rates post-challenge. Similarly, in a study on Nile tilapia by Aly *et al*. (2008), the use of *Bacillus pumilus* and Organic GreenTM significantly improved survival and resistance against the pathogenic bacteria *Aeromonas hydrophila*, aligning with the outcomes of the present research. Additionally, research by Bhatnagar and Lamba (2017) and Bhatnagar and Dhillon (2019) and showed that groups with probiotic bacterial strains in their diet had lower mortality rates during the challenge test with *A. hydrophila*, emphasizing the protective role of probiotics in enhancing fish health and resilience against pathogenic challenges. The study reveals that *L. plantarum* administration on day 30 and *L. pentosus* on day 60 significantly improved growth indicators. Although overall growth indices declined in all groups during the 60 days compared to the first 30 days, there was a notable improvement in the food conversion factor. Interestingly, the combined use of the two probiotics did not exhibit a synergistic effect at the experiment's end, with the growth performance in the combined group being lower than in the individual groups. Notably, *L. pentosus* demonstrated efficacy in reducing fatty liver, possibly through enhancements in hepatocyte cell function, fat metabolism, vitamin/mineral absorption, and reductions in liver glycogen and the liver ALP enzyme. The control treatments showed the highest fatty liver content. Additionally, when subjected to a bacterial challenge with *S. iniae* after 60 days, the control treatment resulted in more significant losses than the groups supplemented with *L. pentosus* and *L. plantarum*, further highlighting their protective effects.

## Conclusions

The study suggests that incorporating *L. plantarum* and *L. pentosus*, as dietary supplements in sea bass feed can significantly enhance growth indicators and the food conversion ratio, making them viable candidates for use in aquaculture practices.

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## Conflicts of interest

The authors declare that they have no conflict of interest.

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