Fishery Technology 62 (2025) : 181 - 191

Inclusion of Probiotics in Feeding Asian Seabass (*Lates calcarifer*) Fingerlings Improves Growth Performance, Body Composition, and Digestive Enzyme Activity

Priya N. P.[1, 2], Shoji Joseph[1], Sibi, K. K.[1] and Anjusoma S.[1, 2]

[1] *ICAR- Central Marine Fisheries Research Institute, Post Box No. 1603, Ernakulam North P.O, Kochi - 682018, Kerala, India*

[2] *Cochin University of Science and Technology, South Kalamassery, Kochi, Kerala 682022*

Abstract

Asian Seabass (*Lates calcarifer*) fingerlings (4.21±0.68 g) were subjected to a 90-day feeding trial to evaluate the effects of various feeds and probiotic supplementation on growth performance, body composition, and digestive enzyme activities under controlled nursery conditions.The fish were fed three experimental diets: Feed CPFA and Feed CPFB, which are Commercial Pellet Feeds (CPF), and Feed C, which is a basal diet containing of trash/ low-valued fish without probiotics. The other treatments included these basal diets supplemented with probiotics - SSP (Single-strain probiotics) and MSP (Multi-strain probiotics). Nine treatments, with three replicates, tested three diets, which included without probiotics, single-strain, and multi-strain probiotics.Fingerlings were stocked at 10 fish per 80-liter tank.The results indicated that growth parameters were significantly influenced ($P<0.05$) by different diets. The fish fed on multi-strain probiotics had significantly higher final body weight (46.17±0.90 g), weight gain (41.07±0.40 g), daily growth rate (0.45±0.00 g/day), specific growth rate (1.42±0.02 g/day), survival rate (100%), and feed conversion ratio than those fed on single-strain probiotics and without probiotics. Statistically significant differences ($P<0.05$) in proximate composition were also observed among the fish-fed different diets. Probiotic feeding improved the protein, fat, and moisture content of Seabass fingerlings. Probiotics also enhanced the activities of digestive enzymes such as amylase, lipase, and protease, and altered the fatty acid profile. These findings highlight the potential of probiotic-enriched diets in enhancing growth, digestion, and nutritional quality in Asian Seabass, supporting their application in sustainable aquaculture practices.

Keywords: Asian Seabass, probiotics, growth performance, body composition, enzyme activity, fattyacid profile.

Introduction

Aquaculture is one of the fastest-growing sectors in global food production (Kong et al., 2020). In developing countries, it plays a crucial role in enhancing food security and providing additional income for fish farmers. The rapid growth of the human population, along with the rising demand for affordable protein sources and the decline in fish catches from natural inland lakes, has intensified the urgent need for the rapid development of aquaculture (El-Saadony et al., 2021). Aquaculture production heavily depends on the external supply of aquafeeds or nutrients to sustain the aquaculture system (Tacon & Metian, 2015). Since nutrition plays a pivotal role in fish production systems, it directly influences the quality of the meat. Advances in nutritional science now allow for the formulation of balanced and comprehensive diets for commercially important species. Adequate nutrient supply, both in terms of quantity and quality, is essential for the growth, health, and reproduction of all cultured species. In aquaculture systems, particularly, feed quality and feeding practices significantly influence sustainability, profitability, and the overall well-being of the species (Syamala, Khandagale, & Dias, 2014).

Received 19 November 2024; Revised 10 February 2025; Accepted 17 February 2025

Handling Editor: Dr. P. S. Shyne Anand

*E-mail: priyanvnraj@gmail.com

Disease outbreaks and growth limitations are major constraints on aquaculture production and trade, negatively impacting economic development in many countries. To address these challenges, various alternative methods have been explored to enhance the quality and sustainability of aquaculture production. Among these methods, probiotics have demonstrated a crucial role in improving aquaculture practices (Hai, 2015). A growing body of research highlights the potential of functional feed additives, such as probiotics, in aquaculture nutrition. Probiotics promote fish growth, enhance digestive enzyme activity, improve serum biochemical and hematological parameters (Dawood,Abo-Al-Ela, & Hasan, 2020), regulate intestinal microbiota, and strengthen immune responses (Yilmaz et al., 2022), including complement activation, lysozyme activity (Ringø et al., 2020), and respiratory burst functions. In aquaculture, probiotics have demonstrated efficacy in improving feed intake, growth performance, and overall health in various species of fish and shrimp (Lara, Olvera-Novoa, Guzmán-Méndez, & López-Madrid, 2003; Das, Lyla, & Khan, 2006). Probiotic supplementation also offers additional benefits, such as improved water quality, enhanced nutrient utilization through supplemental digestive enzyme production, disease mitigation, and increased survival rates (Verschuere,Rombaut, Sorgeloos, & Verstraete, 2000). Probiotic strains like *Lactobacilli* and *Enterococci*, commonly used in terrestrial animal systems, have shown promising results in fish health applications. These findings emphasize the importance of prioritizing research on the appropriate use of probiotics in fish farming systems.

Asian Seabass, a commercially significant food fish, is widely cultivated in cage culture systems in Kerala due to its high market value and adaptability to aquaculture environments. While Asian Seabass farming is widespread in Kerala, farmers face a significant challenge in sourcing adequate and sustainable feed. Currently, the primary feed source for Asian Seabass culture is trash/low-valued fish. During the initial two months of culture, chopped trash/low-valued fish is fed at 10% of the total biomass twice daily, with feeding intensity reduced thereafter. However, concerns over the sustainability of trash/low-valued fish, both in quality and availability, have led to an interest in alternative feed formulations (Syamala et al., 2014). To address these issues, research on alternative feeding options, including various pellet feed formulations and the application of probiotics, is essential. Such studies aim to evaluate the efficacy of different feeds and probiotic treatments in improving growth performance, feed efficiency, and disease resistance. By introducing diverse feed types tailored to Seabass's nutritional requirements, this research could provide farmers with viable alternatives, promoting sustainable and profitable farming practices in the region.

Given the limitations associated with trash/low-valued fish as a feed source and the growing demand for sustainable aquaculture practices, the use of formulated feeds supplemented with probiotics is becoming a necessity. By testing and evaluating different feed formulations and probiotic treatments, this research could lead to the development of reliable and nutritionally balanced feed options, ultimately supporting the adoption of sustainable, profitable Seabass farming practices in Kerala, India.

Material and Methods

Asian Seabass fingerlings (mean weight: 4.21±0.686 g; length: 6.97±0.388 cm) were procured from Rajiv Gandhi Centre for Aquaculture (RGCA), Kochi. The fingerlings were transported in oxygen-filled bags during the evening. Upon arrival, the fish were acclimatized to the laboratory conditions.

Before the feeding trials, the fish were acclimated for one week in the experimental setup. A total of 300 fingerlings were selected and maintained under controlled conditions to minimize stress during acclimation. During this period, the fish were fed a standard commercial diet twice daily to ensure uniform conditioning and adaptation to the experimental environment. This acclimation process aimed to stabilize the physiological condition of the fish and standardize their initial health status for subsequent trials. This standardized acclimation ensured the fingerlings were in optimal health and uniformly prepared for evaluating the effects of different diets and probiotic supplementation.

The experiment used 27 tanks, each with an 80-liter capacity, arranged in a completely randomized design. Nine dietary treatments (T1–T9) were evaluated, with each treatment randomly assigned to three replicate tanks.

The dietary treatments were categorized into three groups based on the inclusion of probiotics, as follows:

1. Basal diets without probiotics
    - o T1: Commercial pellet feed A (CPFA) without probiotics.
    - o T2: Commercial pellet feed B (CPFB) without probiotics.
    - o T3: Trash/low-valued fish (C) without probiotics.
2. Basal diets supplemented with multi-strain probiotics (MSP):
    - o T4: CPFA supplemented with multi-strain probiotics.
    - o T5: CPFB supplemented with multi-strain probiotics.
    - o T6: Trash/low-valued fish supplemented with multi-strain probiotics.
3. Basal diets supplemented with single-strain probiotics (SSP):
    - o T7: CPFA supplemented with single-strain probiotics.
    - o T8: CPFB supplemented with single-strain probiotics.
    - o T9: Trash/low-valued fish supplemented with single-strain probiotics.

This categorization allowed for the assessment of the effects of multi-strain and single-strain probiotic supplementation across different feed types.

The composition of commercial pellet feeds A and B, along with the average proximate composition of trash/low-valued fish, is summarized in Table 1. Probiotic supplementation was applied at 5 g of MSP per kg feed and 100 g of SSP per 5 kg feed, following the manufacturer's specifications. Probiotics were incorporated into the feed using a growth promoter binder or water, uniformly coated onto the feed, and air-dried. Fish were fed twice daily, at around 08:30–09:00 hrs and 17:30–18:00 hrs, at a feeding rate of 10% of body weight. The quantity of feed and probiotics was measured using a calibrated electronic balance (Citizen scale, model CG 2202, S.No. 3140922, capacity: 2200 g, precision: 0.2 g).

At the beginning of the feeding trial, fish were deprived of feed for 24 hours to ensure gut evacuation. Following this, the fish were pooled, and 10 fish were randomly stocked into each experimental tank. To maintain optimal water quality, various parameters were regularly monitored and kept within their respective optimal ranges: pH (7.00), temperature (27°C), dissolved oxygen (DO) (5.50 mg/L), phosphate (0.03 mg/L), nitrite (0.10 mg/L), nitrate (0.50 mg/L), and ammonia (0.01 mg/L).

Water temperature was measured using a thermometer, pH was determined with a pH meter (EutechPC5500, accuracy ±0.01), and dissolved oxygen concentrations were assessed using the titrimetric Winkler method. Nitrite, nitrate, ammonia, and phosphate concentrations were analyzed using spectrophotometric methods. Additionally, 70% of the water in each tank was replaced every alternate day to maintain water quality within the desired parameters.

Fish samples were collected and weighed individually to calculate the growth parameters using the formulae:

Growth parameters

$$\text{Daily growth rate (DGR-g/day)} = \frac{\text{Final weight (g)} - \text{initial weight (g)}}{\text{Culturing days}}$$

Table 1. Proximate composition of commercial pellet feeds "Feed CPFA, Feed CPFB and Feed C" used as food for the Asian Seabass during the experiment.

	Feed	Crude Protein (%)	Crude Fat (%)	Crude fibre (%)	Moisture (%)
1	CPFA	45	10	3.0	12
2	CPFB	44	07	3.0	11
3	Trash/low-value fish	42	09	2.6	08

Specific Growth Rate (SGR-g/day)

$$= \frac{\text{In (Final weight)} - \text{In (Initial weight)}}{\text{Culturing days}}$$

Weight gain (g)

= Final weight – Initial weight

Food Conversion Ratio (FCR)

$$= \frac{\text{Dry food given (kg)}}{\text{Weight gained (kg)}}$$

Survival Rate (%)

$$= \frac{\text{Number of fish harvested}}{\text{Number of fish stocked}} \times 100$$

Biological indices

Hepatosomatic index (HSI) (%)

$$= \frac{\text{Liver wet weight}}{\text{Body wet weight}} \times 100$$

Viscerosomatic index (VSI) (%)

$$= \frac{\text{Visceral wet weight}}{\text{Body wet weight}} \times 100$$

Length-weight relationship (LWR)

Condition factors were estimated by using the equation:

Modified condition factor as $K = 100*W/L^b$

W= total body weight (g), L= total length (cm), b= b value in length-weight relationship.

Fulton's condition factor = $100*W/L^3$

Where W is the weight of the fish in g, L is the total length of the fish in mm, and the factor 100 is used to bring K close to unity.

Relative condition factor = W/aL^b

Where a and b are the exponential form of the logarithmic length-weight equation's intercept and slope.

Fish body composition analyses were performed using the standard methods (AOAC International, 2005) to estimate moisture, crude protein, crude lipid, ash and crude fibre levels. The Kjeldahl method was used to determine the crude protein content of fish. After digestion, crude protein content was analyzed using a semi-automated Kjeldahl system (FOSS Kjeltec 2300). Crude lipid content was determined by the ether-extract method with a Soxhlet system (FOSS Soxtec 2043). The moisture level of the fish was evaluated by oven-drying to constant weight at 60°C. Ash content was determined by incinerating the sample in a muffle furnace at 550°C for 3 hours. Crude fibre content was measured through acid digestion, followed by alkali digestion.

Fish intestinal samples were homogenized using a cold homogenizing buffer before centrifugation. The supernatant was then separated and used to measure the activity of digestive enzymes. Protease activity was analyzed using the casein digestion method (Drapeau, 1976). A mixture of 2.5 ml casein (0.01 N NaOH, 0.05 M tris-phosphate buffer, pH.7.8) and 0.1 ml tissue sample was incubated for 10 min. The reaction was stopped with 10% trichloroacetic acid, filtered, and OD measured at 280 nm.Amylase activity was analyzed using the DNS method (Rick & Stegbauer, 1974). A mixture of 1% starch solution, phosphate buffer (pH 6.9), and 5% tissue homogenate was incubated at 37°C for 30 minutes. After adding DNS, the mixture was heated for 5 min, cooled, and diluted with distilled water, and OD was measured at 540 nm. Lipase activity was estimated using the method of Cherry and Crandall (1932). The tissue homogenate was mixed with distilled water, phosphate buffer, and olive oil, incubated at 37°C for 24h. The mixture was then treated with 95% alcohol and phenolphthalein and titrated with 0.05N NaOH until a permanent pink colour appeared.

The fatty acid profile of fish fed with various diets was analyzed using AOAC official method (AOAC International, 2019). For fatty acid methylation, the sample (approximately 50 mg for oil) was transferred to a glass vial and evaporated to dryness at 40°C in a water bath. The dried sample was treated with 4 ml of 0.5M potassium hydroxide in methanol and heated at 70°C for 2 minutes. Subsequently, 7 ml of 14% boron trifluoride in methanol was added and heated again at 70°C for 5 minutes. After cooling, 5 ml of water, 1 ml of hexane, and 1 g of sodium sulphate were added, followed by shaking. The upper layer was transferred to another vial containing 1 g of Na_2SO_4 and injected into a Gas Chromatography-Flame Ionization Detector. For standard preparation, 1 µl of FLAME MIX standard was directly injected into the system. The sample

weight and extracted fat weight were recorded in an Excel sheet for calculation, with the area percentage from the chromatogram used for comparison.

The normality and homogeneity of variance of the datasets were assessed using the Shapiro-Wilk test and Levene's test, respectively, in SPSS software. Data that did not meet the assumption of normality were analyzed using Kruskal Wallis Test, whereas normally distributed data were subjected to one-way analysis of variance (ANOVA) followed by Tukey's HSD (Honestly Significant Differences) post hoc test. A significance level of 5% ($P<0.05$) was applied to all statistical analyses.

Results & Discussion

When different dietary treatments were administered to Asian Seabass fingerlings, significant variations were observed in growth parameters. Tukey's HSD (Honestly Significant Differences) post hoc test revealed a highly significant effect of different dietary treatments on final weight, weight gain, and daily growth rate of Asian Seabass fingerlings ($P<0.05$). Diet CPFA supplemented with multi-strain probiotics (T4), CPFB supplemented with multi-strain probiotics (T5), trash/low-valued fish supplemented with multi-strain probiotics (T6), CPFA supplemented with single-strain probiotics (T7), and CPFB supplemented with single-strain probiotics (T8) significantly increased FW compared to the trash/low-valued fish without probiotics (T3), with T5 and T6 showing the most positive impact. Diets T6, T5, and T9 exhibited significantly greater weight gain compared to T3, T1, and T2. The Kruskal- Wallis test revealed a significant overall difference in SGR across the different diets ($P=0.008$). However, fish fed diets T5, T4, and T2 attained higher specific growth rates. Fish-fed diet T5 attained a better FCR of 1.41 and a higher survival rate (100%) (Table 2).

The hepatosomatic index (HSI) and viserosomatic index (VSI) of Asian Seabass fish-fed on different diets were significantly different ($P=0.002$) and T2 and T5 showingsignificantly better results compared to other diets. The modified CF, Fulton CF, and relative CF revealed a tendency for an elevated response to probiotic treatment. The probiotic-supplemented diet group exhibited significantly better CF than the other groups (Table 3).

The study results revealed significant variations ($P<0.001$) in dry matter, crude protein, and crude fat content among the diets (Table 4). Fish fed probiotic-supplemented pellet feed (diets T4 and T5) exhibited higher protein content compared to the other treatments. Probiotic supplementation enhances nutrient digestion, absorption, and assimilation,

Table 2. Effect of different feeds and incorporation of probiotics on growth performance, survival rate, and FCR of Asian Seabass

Treatments	Final Weight (g)	Weight Gain(g)	Daily Growth Rate (g/day)	Specific Growth Rate (g/day)	Relative Growth Rate	Survival Rate (%)	FCR
T1	34.35 ± 1.20^{e}	29.37 ± 0.37^{e}	0.326 ± 0.00^{f}	1.00 ± 0.00^{bc}	7.00 ± 0.04^{bc}	80.00 ± 0.00^{a}	1.51 ± 0.01^{b}
T2	34.74 ± 0.84^{e}	31.87 ± 0.11^{f}	0.37 ± 0.00^{g}	1.06 ± 0.03^{d}	7.52 ± 0.24^{cd}	80.00 ± 0.00^{a}	1.40 ± 0.00^{a}
T3	27.08 ± 0.33^{b}	23.57 ± 0.25^{b}	0.26 ± 0.00^{b}	0.98 ± 0.00^{b}	6.70 ± 0.13^{bc}	90.00 ± 0.00^{b}	2.94 ± 0.03^{e}
T4	41.05 ± 0.20^{f}	36.17 ± 0.83^{g}	0.40 ± 0.00^{h}	1.02 ± 0.06^{cd}	7.49 ± 1.07^{cd}	100.0 ± 0.00^{c}	1.50 ± 0.00^{b}
T5	46.17 ± 0.90^{g}	41.07 ± 0.40^{h}	0.45 ± 0.00^{i}	1.42 ± 0.02^{e}	8.06 ± 0.74^{d}	100.0 ± 0.00^{c}	1.41 ± 0.01^{a}
T6	28.80 ± 0.90^{bc}	24.93 ± 0.45^{c}	0.27 ± 0.00^{c}	0.96 ± 0.04^{b}	6.47 ± 0.74^{b}	90.00 ± 0.00^{b}	2.88 ± 0.01^{d}
T7	30.03 ± 0.70^{cd}	26.17 ± 0.41^{c}	0.29 ± 0.00^{d}	0.98 ± 0.02^{bc}	6.74 ± 0.35^{bc}	90.00 ± 0.00^{b}	1.61 ± 0.01^{c}
T8	31.07 ± 0.40^{d}	27.63 ± 0.32^{d}	0.30 ± 0.00^{e}	1.06 ± 0.00^{d}	8.10 ± 0.14^{d}	90.00 ± 0.00^{b}	1.50 ± 0.00^{b}
T9	22.27 ± 0.47^{a}	20.33 ± 0.55^{a}	0.22 ± 0.00^{a}	0.83 ± 0.01^{a}	4.67 ± 0.10^{a}	90.00 ± 0.00^{b}	3.03 ± 0.05^{f}

Results are presented as mean ± SD. Significant differences among feed types were determined using one-way ANOVA ($P<0.05$), followed by Tukey's HSD (Honestly Significant Differences) post hoc test to compare mean values across diets. Mean with different superscript letters in the same column indicate significant differences ($P<0.05$). Values with the same superscripts among different treatments show no significant difference ($P>0.05$).

leading to an improvement in the crude protein content of fish. In contrast, fish fed trash/low-valued fish showed higher fibre and ash content.

Fish in the multi-strain probiotic-supplemented groups (diets T4 and T5) exhibited significantly higher protease activity (Fig. 1) and lipase activity (Fig. 2) compared to other dietary groups. Additionally, amylase activity (Fig. 3) in Asian seabass was significantly elevated in the T4 and T5 groups relative to the other treatments ($P<0.001$). Fatty acid analysis of Asian Seabass fingerlings revealed that the consumption of different diets altered the composition of the fatty acid profile (Table 5). Probiotic fed Seabass had higher values of palmitic acid (Fatty acid C–16: 0), docosahexanoic acid (Fatty acid C–22: 6), and palmitoleic acid (Fatty acid C–16: 1).

Growth performance parameters in aquaculture are strongly influenced by feed quality, quantity, and environmental conditions, making them critical indicators of production yield. This study examined the effects of different feed types and probiotic supplementation on the growth performance, body composition, and digestive enzyme activity of Asian Seabass (*Lates calcarifer*) fingerlings. The observed differences between treatments were analysed by considering the variability within replicates to ensure the robustness of the findings. The results revealed significant differences in performance among dietary treatments, with diet T5 showing the highest efficacy.

Statistical analyses, including ANOVA and Tukey's HSD (Honestly Significant Differences) post hoc test, confirmed that these differences were due to dietary formulations rather than random variation. Pelleted feeds supplemented with probiotics significantly enhanced growth parameters, survival rates, and feed conversion ratios (FCR) compared to non-supplemented treatments. Especially, the multi-strain probiotic-supplemented feed B treatment group demonstrated superior growth indices, underscoring the potential of probiotics to optimize aquaculture practices. These findings are consistent with prior studies (Lee et al., 2021; Eissa et al., 2022; Sokooti, Dezfoulnejad, Baboli, Sary, & Mabudi, 2022), which highlight the positive impact of probiotics on growth performance across various aquatic species. For instance, El-Haroun, Goda, and Chowdhury (2006) reported marked improvements in weight gain, specific growth rate (SGR), and protein efficiency ratio in Nile tilapia fed diets enriched with probiotics.

Our study demonstrates that pellet feed significantly enhances the growth of Asian Seabass. The observed improvements in growth performance, including weight gain and specific growth rate, highlight the effectiveness of formulated pellet feed in meeting the nutritional demands of this species. Ngoh et al. (2015) evaluated the effectiveness of three commercial pelleted feeds for juvenile Asian Seabass through a comprehensive analysis. Their findings revealed significant variations in growth

Table 3. Biological indices and condition factors of Asian Seabass fed different feeds and probiotics

Treatments	Hepatosomatic Index	Viscerosomatic Index	Fultons CF	Relative CF	Modified CF
T1	1.06±0.00^{a}	6.45±0.02^{e}	1.22±0.01^{c}	1.95±0.03^{c}	2.87±0.01^{e}
T2	1.79± 0.04^{f}	6.74±0.02^{g}	1.28±0.05^{e}	1.89±0.03^{b}	2.16±0.02^{d}
T3	1.55±0.03^{d}	5.80±0.09^{c}	1.22±0.00^{c}	1.91±0.04^{b}	1.08±0.04^{a}
T4	1.41±0.01^{c}	6.58±0.01^{f}	1.20±0.07^{b}	1.82±0.05^{a}	1.98±0.05^{c}
T5	1.75±0.04^{ef}	6.59±0.05^{f}	1.22±0.06^{c}	3.07±0.05^{f}	3.72±0.08^{f}
T6	1.05±0.02^{a}	5.03±0.06^{a}	1.22±0.02^{c}	1.82±0.02^{a}	1.97±0.07^{c}
T7	1.20±0.07^{b}	5.52±0.05^{b}	1.17±0.07^{a}	2.05±0.01^{d}	4.45±0.03^{h}
T8	1.72±0.02^{e}	6.49±0.08^{ef}	1.32±0.04^{f}	2.15±0.03^{e}	4.19±0.07^{g}
T9	1.51±0.01^{d}	6.19±0.04^{d}	1.24±0.03^{d}	1.90±0.01^{b}	1.81±0.01^{b}

Results are presented as mean ± SD. Mean with different superscript letters in the same column indicate significant differences ($P<0.05$). Values with the same superscripts among different treatments show no significant difference ($P>0.05$).

performance, nutritional composition, gut morphology and transcriptomic responses among the different diets. Based on these results, the study suggests selecting feeds that not only support optimal growth but also enhance the overall quality of the meat.

Williams et al. (2003) demonstrated that feeding Asian Seabass with a more nutrient dense diet could potentially enhance growth rates. However, their findings suggest that further increase in nutrient intake may lead to excessive lipid deposition, with minimal improvements in somatic growth. This highlights the need for a balanced dietary approach to optimize growth while maintaining overall fish health and product quality. However, the findings of Bunlipatanon, Songseechan, Kongkeo, Abery, and De Silva (2014) suggest a contrasting perspective. Their study on Asian Seabass and tiger grouper culture indicates that there are no distinct environmental, cost-benefit, or resource-use advantages of commercial pellet feeds over traditional trash/low-valued fish.

The present study demonstrated that probiotic-enriched pelleted feeds significantly enhanced the protein, fat, and moisture content of Asian Seabass fingerlings. These findings align with those of Opiyo, Jumbe, Ngugi, and Charo-Karisa (2019), who reported that probiotics improve growth and body composition in Nile tilapia cultured in low-input ponds. The effects of probiotics vary depending on the application levels; for example, *Saccharomyces cerevisiae* exhibited optimal performance at a concentration of 4 g/kg, whereas *Bacillus subtilis* was most effective at 10 g/kg (Abdel-Tawwab, Abdel-Rahman, & Ismael, 2008). Goda et al. (2018) demonstrated that incorporating feed additives such as probiotics, phytobiotics, and exogenous digestive enzymes in weaning diets of European seabass larvae enhanced growth, feed efficiency, survival, and protein deposition, while reducing lipid accumulation.

The ability of *S. cerevisiae* to enhance feed intake and improve body composition highlights its functional significance in aquaculture. In the present study, probiotic supplementation over 90 days increased intestinal lipase, amylase, and protease activity in Seabass fingerlings. Probiotics adhere to the intestinal mucosa and utilize intestinal carbohydrates to produce and synthesize digestive enzymes, thereby improving nutrient digestion and absorption (El-Saadony et al., 2021). This enhanced digestive capacity promotes growth and reduces susceptibility to digestive disorders.

Merrifield, Bradley, Baker, and Davies (2010) reported that probiotics enhance feed digestion by modifying the intestinal environment, a finding further supported by Assan et al. (2022), who observed increased digestive enzyme activity in

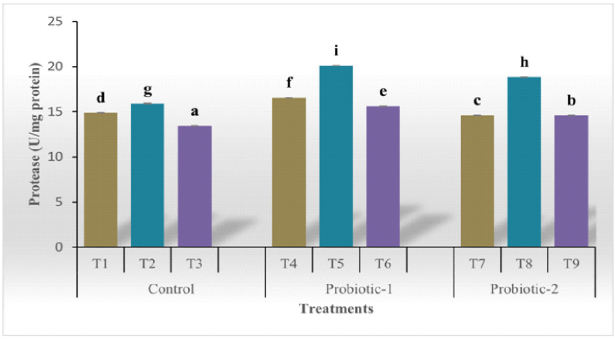

Fig. 1. Protease activity in the intestine of Asian Seabass fed with different diets. Mean±SD with different superscripts is significantly different (P<0.05)

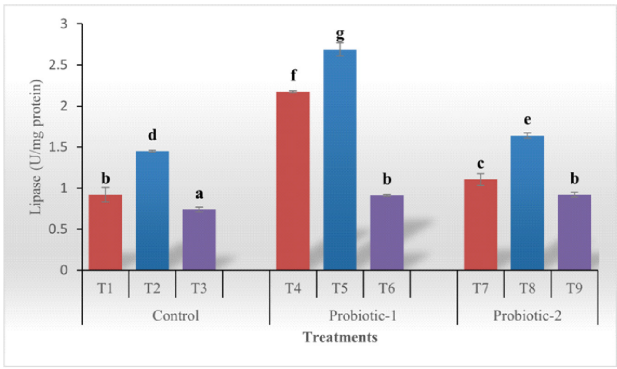

Fig. 2. Lipase activity in the intestine of Asian Seabass fed with different diets. Mean±SD with different superscripts is significantly different (P<0.05)

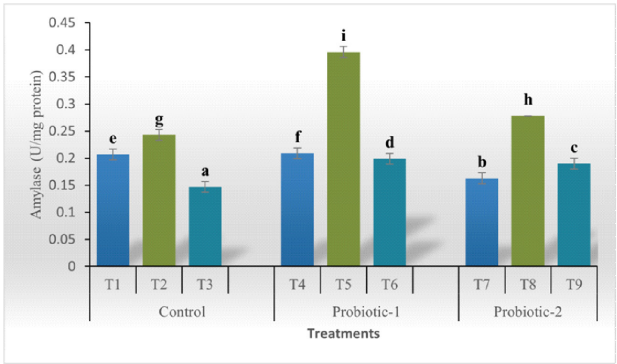

Fig. 3. Amylase activity in the intestine of Asian Seabass fed with different diets. Mean ± SD with different superscripts is significantly different (P<0.05)

Table 4. Proximate composition of Asian Seabass fed different feeds supplemented with probiotics

	Crude Protein (%)	Crude Fat (%)	Crude Ash (%)	Moisture (%)	Dry Matter	Crude Fibre (%)
T1	54.47±0.06^{ab}	14.85±0.20^{a}	14.53±0.27^{b}	5.52±0.39^{b}	94.47±0.39^{d}	1.99±0.04^{g}
T2	55.44±0.15^{d}	21.60±0.12^{e}	15.36±0.09^{cd}	9.40±0.35^{e}	90.60±0.35^{a}	1.82±0.02^{f}
T3	54.42±0.17^{a}	15.78±0.16^{b}	17.54±0.20^{e}	7.21±0.13^{d}	92.79±0.13^{b}	2.46±0.01^{h}
T4	55.58±0.20^{d}	19.38±0.34^{d}	14.60±0.20^{b}	3.31±0.14^{a}	96.69±0.14^{f}	1.45±0.01^{e}
T5	56.28±0.05^{e}	21.17±0.27^{e}	15.50±0.14^{d}	5.36±0.28^{b}	95.02±0.06^{e}	1.07±0.00^{d}
T6	54.80±0.15^{bc}	17.38±0.32^{c}	15.62±0.11^{d}	7.417±0.36^{d}	92.58±0.36^{b}	0.33±0.00^{b}
T7	54.70±0.06^{abc}	19.10±0.44^{d}	13.56±0.15^{a}	5.50±0.41^{b}	94.50±0.41^{d}	1.42±0.01^{e}
T8	54.92±0.33^{c}	17.73±0.21^{c}	15.17±0.04^{c}	5.38±0.29^{b}	94.62±0.29^{de}	0.77±0.00^{c}
T9	54.65±0.28^{abc}	17.29±0.64^{c}	15.15±0.03^{c}	6.57±0.20^{c}	93.43±0.20^{c}	0.22±0.00^{a}

Results are presented as mean ± SD. Significant differences among feed types. Mean with different superscript letters in the same column indicate significant differences($P<0.05$). Values with the same letters among different treatments show no significant difference ($P>0.05$).

various aquatic species. Supporting these findings, studies on *Oreochromis niloticus* (Liu et al., 2017) and prawns (*Penaeus vannamei*) (Wang, 2007) have demonstrated that probiotics, particularly *Bacillus* spp., significantly enhance enzyme activity, thereby promoting more efficient feed digestion and utilization.

The fatty acid profiles of Seabass in this study showed notable variations based on feed types and probiotic supplementation. Dietary supplementation with probiotics can alter the fatty acid composition of fish by influencing lipid metabolism, enhancing nutrient utilization, and modulating gut microbiota. Probiotics improve the digestion and absorption of dietary lipids by increasing lipase enzyme activity, leading to better fatty acid assimilation. Probiotic inclusion influenced the synthesis of essential nutrients, such as fatty acids and vitamins, enhancing their bioavailability (Vine et al., 2004). This finding aligns with previous research that demonstrated a strong correlation between dietary composition and fatty acid profiles (Mourente & Bell, 2006; Arslan, Sirkecioglu, Bayir, Arslan, & Aras, 2012).

Asian Seabass (*Lates calcarifer*) farming in Kerala is a growing aquaculture practice, yet it faces a critical obstacle due to the lack of adequate and sustainable feed options. Currently, farmers rely on trash/low-valued fish—a low-cost but nutrient-inconsistent feed source—leading to variable growth rates and suboptimal health of the Seabass stock. This variability hampers the productivity and profitability of seabass farming. Addressing this issue requires research into alternative, nutrient-stable feeding strategies, including specially formulated pellet feeds and probiotics. The rising costs of Asian Seabass feed present a significant challenge to the growth and profitability of seabass production. This study underscores the importance of identifying cost-effective and nutritionally balanced feed and supplements to enhance growth performance and sustainability in aquaculture. The present results highlight that the integration of probiotics with pelleted feeds enhances growth performance, survival rates, and nutrient utilization in Asian Seabass. These findings contribute to the growing body of evidence supporting the use of probiotics as an effective and sustainable strategy for improving aquaculture practices. Future research should focus on optimizing probiotic dosages and formulations to maximize their benefits in diverse aquaculture systems.

The findings of the study indicated that Asian Seabass fed commercial pellet feeds supplemented with multi-strainprobiotic diet showed significantly higher growth performance than other treatment groups. Fish in the multi-strain probiotics incorporated into commercial pellet feed B diet group demonstrated considerably superior proximate composition. Additionally, dietary interventions had a significant influence on digestive enzyme activities, with probiotic supplementation significantly increasing these enzyme activities. The results further highlight that dietary interventions were influenced by the composition of individual fatty acids, with

Table 5. Fatty acid profile of Asian Seabass fed with different feeds and probiotics.

	Fatty acid profile	T1	T2	T3	T4	T5	T6	T7	T8	T9
Saturated Fatty Acid (SFA)										
1	Butyric acid C-4 :0	N.D.	0.02±0.00	N.D.	1.2±0.01	1.80±0.03	1.00±0.01	0.60±0.04	1.00±0.01	0.04±0.03
2	Lauric acid C-12 :0	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	2.00±0.03	1.80±0.02	1.30±0.01
3	Myristic acid C-14:0	N.D.	N.D.	N.D.	2.60±0.02	3.00±0.01	2.20±0.05	5.50±0.02	5.60±0.06	5.10±0.04
4	Palmitic acid C-16:0	26.60±0.1	25.80±0.13	24.00±0.15	34.20±0.11	36.10±0.09	30.20±0.07	42.60±0.05	43.80±0.04	40.90±0.03
5	Heptadecanoic acid C-17:0	N.D.	N.D.	N.D.	0.03±0.00	0.05±0.00	0.02±0.01	0.70±0.03	0.70±0.04	0.60±0.02
6	Stearic acid C-18:0	19.90±0.02	19.90±0.03	19.20±0.01	9.40±0.03	8.80±0.02	8.30±0.05	9.90±0.07	10.20±0.01	9.10±0.00
7	Arachidic acid C-20:0	3.60±0.03	3.20±0.02	3.10±0.01	1.20±0.05	1.00±0.03	0.90±0.05	0.80±0.07	0.90±0.03	0.70±0.01
Total SFA		50.10	48.92	46.30	48.63	50.75	42.62	62.10	64.00	57.74
Monounsaturated Fatty Acids (MUFA)										
8	Palmitoleic acid C-16:1	N.D.	N.D.	N.D.	5.00±0.05	5.30±0.02	4.90±0.01	4.70±0.05	5.30±0.02	4.40±0.03
9	Oleic acid C-18:1	41.70±0.04	42.70±0.1	40.90±0.11	27.00±0.09	25.30±0.05	22.50±0.03	29.10±0.07	27.00±0.09	24.30±0.03
10	Fatty acid C-22:1	N.D.	N.D.	N.D.	1.60±0.04	1.70±0.04	1.50±0.09	N.D.	N.D.	N.D.
Total MUFA		41.70	42.70	40.90	33.60	32.30	28.90	33.80	32.30	28.70
Polyunsaturated Fatty Acids (PUFA)										
11	Linolenic acid C-18:2	2.60±0.04	2.80±0.01	2.40±0.05	4.80±0.03	4.30±0.07	4.10±0.04	3.30±0.05	2.70±0.02	2.50±0.03
12	Eicosatrienoic acid C-20:3	2.20±0.01	2.50±0.05	2.00±0.06	1.40±0.02	1.30±0.05	1.10±0.02	1.20±0.01	1.30±0.04	1.00±0.04
13	Eicosapentaenoic C-20:5 -	3.50±0.04	3.10±0.04	2.90±0.01	1.00±0.07	0.80±0.05	0.60±0.06	0.40±0.08	0.50±0.01	0.30±0.04
14	Docosahexanoic acid C-22:6	7.00±0.03	7.90±0.02	5.03±0.02	11.90±0.06	12.30±0.07	11.20±0.03	1.10±0.02	1.30±0.07	1.00±0.01
15	Arachidonic acid C-20:4	1.80±0.04	1.90±0.01	1.00±0.02	2.30±0.04	2.40±0.02	2.10±0.01	1.20±0.08	1.40±0.06	1.10±0.02
Total PUFA		17.10	18.20	13.03	21.40	21.10	19.10	7.20	7.20	5.90

Mean values with ± SD are represented. N.D.: not detected

palmitic acid being the predominant saturated fatty acid. Furthermore, the use of pellet feed and probiotics offers substantial benefits by optimizing fish growth and improving water quality, thereby reducing the environmental impact of aquaculture. Based on these findings, it is recommended that commercial pellet feed (CPFB) supplemented with a multi-strain probiotic be utilized for the optimal growth and health of Asian Seabass fingerlings in nursery conditions. The cost of feed is an important factor for Asian Seabass production. Therefore, it is necessary to investigate alternative sources of inexpensive, balanced feeds to promote our local industry and increase the profitability of Seabass production. Future research should prioritize elucidating the long-term effects of probiotics in cage culture, focusing primarily on the underlying physiological, microbial and immunological mecha-

nisms driving the improvements, as well as enhancing their validation and optimize their application in sustainable aquaculture.

Acknowledgments

The authors express their heartfelt gratitude to Dr. Gopalakrishnan, Former Director of CMFRI, for providing the necessary facilities to carry out this research. We are also deeply thankful to the scientists and technical staff of the Mariculture Division, CMFRI, Kochi, for their invaluable support and assistance during the study. Additionally, we extend our sincere thanks to the CSIR for their financial support.

References

Abdel-Tawwab, M., Abdel-Rahman, A. M., & Ismael, N. E. M.(2008). Evaluation of commercial live bakers' yeast, *Saccharomyces cerevisiae* as a growth and immunity promoter for fry Nile tilapia, *Oreochromis niloticus* (L.) challenged *in situ* with *Aeromonas hydrophila*. *Aquaculture, 280*(1-4),185-189.https://doi.org/10.1016/j.aquaculture.2008.03.055.

AOAC International. (2005). Official method 934.01. *Official methods of analysis of AOAC International*(18thed.). AOAC International, Gaithersburg, MD.

AOAC International. (2019). Official Method 969.33. *Official Methods of Analysis of AOAC International* (21st ed.). AOAC International, Gaithersburg, MD.

Arslan, M., Sirkecioglu, N., Bayir, A., Arslan, H., & Aras, M.(2012). The influence of substitution of dietary fish oil with different vegetable oils on performance and fatty acid composition of brown trout, *Salmo trutta*. *Turkish Journal of Fisheries and Aquatic Sciences, 12*(3), 575-583. https://doi.org/10.4194/1303-2712-v12-3-04.

Assan, D., Kuebutornye, F. K. A., Hlordzi, V., Chen, H., Mraz, J., Mustapha, U. F., &Abarike, E. D. (2022). Effects of probiotics on digestive enzymes of fish (finfish and shellfish); status and prospects: a mini-review. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 257*, Article 110653. https://doi.org/10.1016/j.cbpb.2021.110653.

Bunlipatanon, P., Songseechan, N., Kongkeo, H., Abery, N. W., & De Silva, S. S. (2014). Comparative efficacy of trash fish versus compounded commercial feeds in cage aquaculture of Asian seabass (*Lates calcarifer*)(Bloch) and tiger grouper (*Epinephelus fuscoguttatus*)(Forsskål). *Aquaculture Research, 45*(3), 373-388. https://doi.org/10.1111/j.1365-2109.2012.03234.x.

Cherry, I.S., & Crandall Jr., L.A. (1932). The specificity of pancreatic lipase: its appearance in the blood after pancreatic injury. *American Journal of Physiology, 100*(2), 266-273.https://doi.org/10.1152/ajplegacy.1932.100.2.266.

Das, S., Lyla, P. S., & Khan, S. A. (2006). Application of Streptomyces as a probiotic in the laboratory culture of *Penaeus monodon* (Fabricius). *Israeli Journal of Aquaculture, 58*(3), 198-204.

Dawood, M. A. O., Abo-Al-Ela, H. G., & Hasan, M. T. (2020). Modulation of transcriptomic profile in aquatic animals: Probiotics, prebiotics and synbiotics scenarios. *Fish &Shellfish Immunology, 97*, 268-282.https://doi.org/10.1016/j.fsi.2019.12.054.

Drapeau, G. R. (1976). Protease from *Staphylococcus aureus*. *Methods in Enzymology, 45*, 469-475. https://doi.org/10.1016/S00 76-6879(76)45041-3.

Eissa, E. H., Baghdady, E. S., Gaafar, A. Y., El-Badawi, A. A., Bazina, W. K., Abd Al-Kareem, O. M., & Abd El-Hamed, N. N. B. (2022). Assessing the influence of dietary *Pediococcusacidilactici* probiotic supplementation in the feed of European sea bass (*Dicentrarchuslabrax* L.) (Linnaeus, 1758) on-farm water quality, growth, feed utilization, survival rate, body composition, blood biochemical parameters, and intestinal histology. *Aquaculture Nutrition, 2022*(1), Article 5841220. https://doi.org/10.1155/2022/5841220.

El Haroun, E. R., Goda, A. M., & Chowdhury, M. A. K.(2006). Effect of dietary probiotic Biogen® supplementation as a growth promoter on growth performance and feed utilization of Nile tilapia *Oreochromis niloticus* (L.). *Aquaculture Research, 37*(14), 1473-1480. https://doi.org/10.1111/j.1365-2109.2006.01584.x.

El-Saadony, M. T., Alagawany, M., Patra, A. K., Kar, I., Tiwari, R., Dawood, M. A. O.,Dhama, K.,& Abdel-Latif, H. M. R.(2021). The functionality of probiotics in aquaculture: An overview. *Fish &Shellfish Immunology, 117*, 36-52. https://doi.org/10.1016/j.fsi.2021.07.007.

Goda, A. M., Omar, E. A., Srour, T. M., Kotiet, A. M., El-Haroun, E., & Davies, S. J. (2018). Effect of diets supplemented with feed additives on growth, feed utilization, survival, body composition and intestinal bacterial load of early weaning European seabass, *Dicentrarchuslabrax* post-larvae. *Aquaculture International, 26*, 169-183. https://doi.org/10.1007/s10499-017-0200-8.

Hai, N. V. (2015). The use of probiotics in aquaculture. *Journal of Applied Microbiology, 119*(4), 917-935. https://doi.org/10.1111/jam.12886.

Kong, W., Huang, S., Yang, Z., Shi, F., Feng, Y., & Khatoon, Z. (2020). Fish feed quality is a key factor in impacting aquaculture water environment: evidence from incubator experiments. *Scientific reports, 10*(1), Article 187. https://doi.org/10.1038/s41598-019-57063-w.

Lara-Flores, M., Olvera-Novoa, M. A., Guzmán-Méndez, B. E., & López-Madrid, W. (2003). Use of the bacteria *Streptococcus faecium* and *Lactobacillus acidophilus*, and the yeast *Saccharomyces cerevisiae* as growth promoters

in Nile tilapia (*Oreochromis niloticus*). *Aquaculture*, *216*(1-4), 193-201.https://doi.org/10.1016/S0044-8486(02)00277-6.

Lee, C., Kim, S., Shin, J., Kim, M. G., Gunathilaka, B. E., Kim, S. H., Kim, J. E., Ji, S. C., Han, J. E.,& Lee, K. J. (2021). Dietary supplementations of *Bacillus* probiotic improve digestibility, growth performance, innate immunity, and water ammonia level for Pacific white shrimp, *Litopenaeusvannamei*. *Aquaculture International*, *29*(6), 2463-2475. https://doi.org/10.1007/s10499-021-00760-z.

Liu, H., Wang, S., Cai, Y., Guo, X., Cao, Z., Zhang, Y., Liu, S., Yuan, W., Zhu, W., Zheng, Y., Xie, Z.., Guo, W.,& Zhou, Y. (2017). Dietary administration of *Bacillus subtilis* HAINUP40 enhances growth, digestive enzyme activities, innate immune responses, and disease resistance of tilapia, *Oreochromis niloticus*. *Fish &Shellfish Immunology*, *60*, 326-333. https://doi.org/10.1016/j.fsi.2016.12.003.

Merrifield, D. L., Bradley, G., Baker, R. T. M., & Davies, S. J. (2010). Probiotic applications for rainbow trout (*Oncorhynchus mykiss* Walbaum) II. Effects on growth performance, feed utilization, intestinal microbiota and related health criteria postantibiotic treatment. *Aquaculture Nutrition*, *16*(5), 496-503. https://doi.org/10.1111/j.1365-2095.2009.00688.x.

Mourente, G., & Bell, J. G. (2006). Partial replacement of dietary fish oil with blends of vegetable oils (rapeseed, linseed, and palm oils) in diets for European sea bass (*Dicentrarchuslabrax* L.) over a long-term growth study: effects on muscle and liver fatty acid composition and effectiveness of a fish oil finishing diet. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *145*(3-4), 389-399. https://doi.org/10.1016/j.cbpb.2006.08.012.

Ngoh, S. Y., Tan, D., Shen, X., Kathiresan, P., Jiang, J., Liew, W. C.,Thevasagayam, N. M., Kwan, H. Y., Saju, J. M., Prakki, S. R. S., Goh, C. H., Wong, H. C., Chan, T. T., Mézes, M.,& Orban, L. (2015). Nutrigenomic and nutritional analyses reveal the effects of pelleted feeds on Asian Seabass (*Lates calcarifer*).*PloS One*,*10*(12), Articlee0145456.https://doi.org/10.1371/journal.pone.0145456.

Opiyo, M. A., Jumbe, J., Ngugi, C. C., & Charo-Karisa, H. (2019). Different levels of probiotics affect the growth, survival, and body composition of Nile tilapia (*Oreochromis niloticus*) cultured in low-input ponds. *Scientific African*, *4*, Article e00103.https://doi.org/10.1016/j.sciaf.2019.e00103.

Rick, W., & Stegbauer, H. P. (1974). á-Amylase measurement of reducing groups. In H. U. Bergmeyer, & K. Gawehn (Eds.), *Methods of enzymatic analysis*(pp. 885-915). Academic Press Inc., New York.

Ringø, E., Van Doan, H., Lee, S. H., Soltani, M., Hoseinifar, S. H., Harikrishnan, R., & Song, S. K. (2020). Probiotics, lactic acid bacteria, and bacilli: interesting supplementation for aquaculture. *Journal of Applied Microbiology*, *129* (1), 116-136. https://doi.org/10.1111/jam.14628.

Sokooti, R., Dezfoulnejad, M. C., Baboli, M. J., Sary, A. A., &Mabudi, H. (2022). The effects of probiotics supplemented diets on Asian sea bass (*Lates calcarifer*): Growth performance, microbial flora, digestive enzymes activity, serum biochemical and non specific immune indices. *Aquaculture Research*, *53*(16), 5500-5509. https://doi.org/10.1111/are.16032.

Syamala, K., Khandagale, P. A., & Dias, J. R. (2014). Feed management in cage culture.*Training manual on "Cage Culture of Marine Fin fish and Shell fish in Open Sea"* (pp. 35-43). Central Marine Fisheries Research Institute, Kochi.

Tacon, A. G., & Metian, M. (2015). Feed matters: satisfying the feed demand of aquaculture. *Reviews in Fisheries Science& Aquaculture*, *23*(1), 1-10. https://doi.org/10.1080/23308249.2014.987209.

Verschuere, L., Rombaut, G., Sorgeloos, P.,& Verstraete, W. (2000). Probiotic bacteria as biological control agents in aquaculture. *Microbiology and Molecular Biology Reviews,64*, 655–671. https://doi.org/10.1128/mmbr.64.4.655-671.2000.

Vine, N. G., Leukes, W. D., Kaiser, H., Daya, S., Baxter, J.,& Hecht, T.(2004). Competition for attachment of aquaculture candidate probiotic and pathogenic bacteria on fish intestinal mucus.*Journal ofFish Diseases,27*(6), 319–326. https://doi.org/10.1111/j.1365-2761.2004.00542.x.

Wang, Y. B. (2007). Effect of probiotics on growth performance and digestive enzyme activity of the shrimp *Penaeus vannamei*. *Aquaculture*, *269*(1-4), 259-264.https://doi.org/10.1016/j.aquaculture.2007.05.035.

Williams, K. C., Barlow, C. G., Rodgers, L., Hockings, I., Agcopra, C., & Ruscoe, I. (2003). Asian seabass (*Lates calcarifer*) perform well when fed pelleted diets high in protein and lipid. *Aquaculture*, *225*(1-4), 191-206.https://doi.org/10.1016/S0044-8486(03)00278-3.

Yilmaz, S., Yilmaz, E., Dawood, M. A. O., Ringø, E., Ahmadifar, E., & Abdel-Latif, H. M. R.(2022). Probiotics, prebiotics, and synbiotics used to control vibriosis in fish: A review. *Aquaculture*, *547*, Article 737514.https://doi.org/10.1016/j.aquaculture.2021.737514.