



Improvement of brown shrimp (*Penaeus aztecus*) culture parameters through dietary enriched synbiotic in a biofloc system

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Abstract

This study aimed to determine the potential efficacy of dietary prebiotics, probiotics, and synbiotic on growth performance, total hemocyte count (THC), hepatopancreas histology, and gut microbiota composition of brown shrimp (*Penaeus aztecus*) under biofloc technology (BFT) conditions for 84 days. Seven feed additive groups were formed as C: control group with no feed additive, P: probiotic mixture (1 mL, 1×10^9 CFU/100 g feed, *Lactobacillus plantarum*, *L. acidophilus*, *L. salivarius*, and *Bacillus subtilis*), A: 0.2 g *Arthrospira*/100 g feed, S: P + A, ES1: P + A + 0.1 g MOS/100 g feed, ES2: P + A + 0.2 g MOS/100 g feed, ES3: P + A + 0.3 g MOS/100 g feed. At the end of the trial, P, A, and S showed an intermediate effect on final body weight (FBW) compared to the control group, while FBW significantly increased in the ES1, ES2, and ES3 groups ($P < 0.05$). Gut microbiota diversity revealed a predominance of Proteobacteria phylum and a decrease in Bacillota phylum in dietary prebiotic, probiotic, and synbiotic treatments. The number of B cells increased in the hepatopancreas of brown shrimp fed diets containing feed additives, indicating a significant effect on hepatopancreatic tubules. THC levels of the shrimps in the experimental groups increased, and the highest values were reached in shrimps fed with synbiotic diets ($P < 0.05$). In conclusion, it can be concluded that enriched synbiotic diets supplemented with probiotic mixture, *Arthrospira*, and mannan oligosaccharide can promote healthy growth for brown shrimp under BFT conditions.

Keywords Microbiota · Growth · Brown shrimp · Synbiotic · Biofloc

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Introduction

Global seafood demand has surged unprecedentedly in the last two decades, leading to a rise in culture production that has surpassed that of capture fisheries (FAO 2022). However, there are questions about the sustainability of the supply. By 2050, the world population is expected to reach nearly 10 billion, resulting in the need for almost 300 million tons more high-quality protein (Tacon et al. 2022; Khanjani et al. 2023). Challenges such as global climate change scenarios and environmental degradation also pose vital risks to food security (Addo et al. 2023; Sun et al. 2023). Aquaculture is one of the critical potentials for maintaining the resilience of global demand in the face of these growing challenges. Globally, aquaculture production has recorded by 500% since the late 1980s, making it an important contributor to achieving the United Nations Sustainable Development Goals (Jiang et al. 2022). Despite its strong global potential, aquaculture is associated with the overconsumption or pollution of water (Meng and Feagin 2019; MacLeod et al. 2020) and the intensive use of antibiotics (Li et al. 2021; Adenaya et al. 2023). In this context, the aquaculture industry must adopt sustainable and environmentally friendly production strategies to meet future food demand.

Shrimp farming is one of the main sectors in aquaculture, accounting for the majority of production among crustacean species (Addo et al. 2023). Pacific white shrimp (*Litopenaeus vannamei*), the most important species cultivated, accounts for 80% of total shrimp farming, with a production of 4.4 million tons (FAO 2022). Just as the over-intensified production of tiger shrimp (*Penaeus monodon*) in the 90 s led to the white spot syndrome virus disease outbreak, today's intensive Pacific white shrimp farming has also resulted in significant disease outbreaks (Panigrahi et al. 2023). Disease-related problems will likely deepen in shrimp farming, which is expected to increase further in the near future (El-Saadony et al. 2022). It is known that there are no effective treatments for these major diseases in shrimp farming and the most appropriate approach is functional feed additives such as prebiotics and probiotics to promote healthy growth (Rezende et al. 2022). Also, ensuring species diversity for sustainable shrimp culture and domestication of naturally distributed species with aquaculture potential can play a role in overcoming these problems. The selection of aquatic species suitable for aquaculture depends primarily on their presence in the region's natural fauna. When a species successfully establishes a population in a particular region, it indicates that the species has adapted to the local environment, making it a valuable candidate for alternative aquaculture practices.

Native to the Western Atlantic, the brown shrimp (*Penaeus aztecus*) is a penaeid shrimp highly valued for its commercial importance (Al-Badran et al. 2019). The initial sighting of the brown shrimp in the Mediterranean Sea was documented in 2009 (Deval et al. 2010), followed by reports from the Eastern Mediterranean coast, Turkey (Gökoğlu and Özvarol 2013; Stergiou et al. 2014). Studies conducted with brown shrimp showed promise as an alternative aquaculture species (Genc et al. 2024a; 2024b).

Biofloc technology (BFT) as an environmentally friendly application has been the subject of numerous studies, with a particular interest in different shrimp species, and has proven its effectiveness on functions such as growth performance, disease resistance, minimum water use, and yield per unit area (Emerenciano et al. 2012; Ekasari et al. 2014; Zhao et al. 2016; Kaya et al. 2020; Khanjani et al. 2022). BFT, which converts all wastes into microbial nutrients, is valuable in improving feed utilization and reducing the feeding rate for shrimp (Nisar et al. 2022). BFT is an application that can improve aquaculture efficiency by enriching the digestive tract microbiota composition with feed additives with

functional properties to induce healthy culture (Qiu et al. 2023) and has become one of the focus culture techniques due to its sustainable approaches (Khanjani et al. 2022; Yu et al. 2024). Recently, the use of prebiotics (Rodrigues et al. 2018; Van Doan et al. 2019; 2021) and probiotics (Mohammadi et al. 2020; He et al. 2023) to enhance the efficacy of BFT has been focused on. Prebiotics are indigestible feed ingredients that can improve growth and microbiota in the digestive tract. Prebiotics promote healthy gut microbiota by resisting acidity and digestive enzymes, and they nourish gut microbes, support fermentation, and produce vitamins, short-chain fatty acids, and other beneficial compounds (Ramasubburayan et al. 2025). After being the subject of scientific research for many years regarding prebiotic properties, one of the aquaculture industry's feed additives with the highest commercial use and accessibility in every part of the world is mannan oligosaccharide (MOS), obtained from the cell wall of baker's yeast (del Valle et al. 2023). Genc et al. (2024b) reported positive effects of dietary MOS supplementation on brown shrimp production parameters under BFT conditions. Microalgae also serve as prebiotics in aquaculture feeds (Nagappan et al. 2021). Microalgae can support improved growth performance and stronger immunity in the diet (Zhang et al. 2025). Microalgae are used as supplements in aquaculture feeds because they are rich in protein, carotenoids, vitamins, and essential fatty acids and are nutritious and economical (Belay et al. 1996; Kiron et al. 2012). One of the most common microalgae is *Arthrospira (Spirulina) platensis*, which has functional properties (Liu et al. 2022). *Arthrospira platensis*-based supplements promote health through immunomodulatory, antioxidant, anticancer, antimicrobial, and antiviral effects (Sotiroudīs et al. 2013). *Arthrospira platensis* has been successfully applied in aquaculture. For example, diets containing *A. platensis* improved the health of fish (Mahmoud et al. 2018) and shrimp (Chen et al. 2016). Probiotics are living microorganisms known to provide beneficial effects on the health of the host. Probiotics benefit the host by suppressing pathogens, supporting digestion, boosting immunity, and enhancing antiviral effects (Muthu et al. 2024). *Lactobacillus* spp. and *Bacillus* spp. are the most widely used probiotics in many cultured species, including shrimp (El-Saadony et al. 2021). Numerous studies have reported that these beneficial bacteria, which typically belong to genera such as *Lactobacillus* and *Bacillus*, improve growth and health conditions in aquatic organisms (Pavadi et al. 2018; Kewcharoen and Srisapoom 2019; Mohamad et al. 2020; Ringø et al. 2020). Although the effects of prebiotics and probiotics are usually evaluated separately, promising studies on their combined use (synbiotic) have recently been increasing, showing that synbiotic application provides more benefits to the host (Dawood et al. 2020; Yao et al. 2021; Hersi et al. 2023). However, there needs to be more knowledge regarding the effects of synbiotic under BFT conditions in brown shrimp culture. Therefore, the aim of this study was to determine the effects of MOS, *Arthrospira*, and probiotics in diet, both individually and in combination as synbiotic, on the growth efficiency of brown shrimp (*P. aztecus*).

Material and methods

Experimental animals and conditions

The feeding trial was conducted at the Fisheries Unit of Agricultural Applications and Research Center (Faculty of Agriculture, Tokat Gaziosmanpaşa University, Tokat, Turkey). Four hundred and twenty juvenile brown shrimp (*Penaeus aztecus*) were obtained

from Iskenderun Technical University, Faculty of Marine Sciences Research Unit, and were transferred to the Fisheries Unit. Shrimps were acclimatized with basal diet for 15 days before the trial. After acclimation, juvenile shrimps with an average initial weight of 1.21 ± 0.01 g were stocked into 21 plastic tanks (water capacity 40 L, 40 cm water depth) in triplicate with 20 individuals per tank. For the BFT application, experimental tanks were prepared with artificial seawater (ReeFlowers Caledonia Reef Salt, Basaksehir, Turkey) containing pre-produced biofloc (C:N ratio of 15:1) (Avnimelech 2012). Sugar beet molasses (*Beta vulgaris*) was used as a carbon source for each BFT treatment during the trial. The experimental tanks were controlled daily with a refractometer to maintain optimum salinity (30 ppt). All experimental tanks were compensated for increased salinity in evaporation losses with good quality freshwater obtained by reverse osmosis.

Experimental diet preparation and feeding trial

Five diets were formulated to contain approximately 38% crude protein (Supplementary File, Table S1). Seven feed additive groups were formed, including the control, probiotics, prebiotics, and synbiotic. The commercial probiotic mixture (Olvit probiotic, Humat Chemical, Kocaeli, TR), spirulina (*Arthrospira platensis*), and mannan oligosaccharide (Biomos, Altech, TR) were applied as feed additives. Dried *Arthrospira* was obtained from the algae production laboratory at Yalova University. The experimental groups were as follows:

- C control group with no feed additive.
- P probiotic mixture (1 mL, 1×10^9 CFU/100 g feed, *Lactobacillus plantarum*, *L. acidophilus*, *L. salivarius*, and *Bacillus subtilis*, in the commercial solution, each probiotic bacterial species is represented at a concentration of 2.5×10^8 CFU/mL, with a final probiotic concentration of 1×10^9 CFU/ml; this solution has been applied to feed at a rate of 1×10^9 CFU/100 g of feed.)
- A 0.2 g *Arthrospira*/100 g feed.
- S probiotic mixture (1 mL, 1×10^9 CFU/100 g feed) + 0.2 g *Arthrospira*/100 g feed.
- ES1 probiotic mixture (1 mL, 1×10^9 CFU/100 g feed) + 0.2 g *Arthrospira*/100 g feed + 0.1 g MOS/100 g feed.
- ES2 probiotic mixture (1 mL, 1×10^9 CFU/100 g feed) + 0.2 g *Arthrospira*/100 g feed + 0.2 g MOS/100 g feed.
- ES3 probiotic mixture (1 mL, 1×10^9 CFU/100 g feed) + 0.2 g *Arthrospira*/100 g feed + 0.3 g MOS/100 g feed.

Arthrospira and MOS doses were added to the basal diet in dry powder form, and then, pellets were prepared. For group P, the probiotics (1 mL probiotics solution + 9 mL sterile saline) were added to 100 g of basal feed by spraying. For group S, the probiotics were sprayed on diet A; for the ES1, ES2, and ES3 groups, the solution was sprayed similarly. Moreover, 10 mL of sterile saline was applied to 100 g of basal diet for the control group. Then, the diets were air-dried and preserved at -20 °C until use. The shrimp were fed 6% of body weight twice daily and gradually reduced to 3% at the end of the experiment.

Sampling

After 84 days (12 weeks as sufficient time to observe the effects of feed additives on the growth, health, and overall performance of shrimps) of the feeding trial, shrimps were starved for 24 h and weighed for growth parameters. Three shrimps were sampled per tank and stored at $-20\text{ }^{\circ}\text{C}$ until the whole-body proximate analysis. The posterior digestive contents of three shrimp were collected from each tank (combined into one sample for each group) to perform bacterial diversity analyses.

Water quality

Water temperature, dissolved oxygen, pH, and salinity were measured daily during the study. Alkalinity, calcium, phosphate, total ammonia–nitrogen, total nitrate–nitrogen, and total nitrite–nitrogen levels were recorded at weekly intervals (Iris Visible Spectrophotometer, HI801-01 model, Hanna Instruments, USA).

Growth performance

After the rearing period, shrimp performance parameters were determined as follows:

Weight gain (WG; g/shrimp) = Final weight (g) – Initial weight (g).

Daily weight gain (DWG; g/shrimp) = (Final weight (g) – Initial weight (g))/days.

Weekly weight gain (WWG; g/shrimp) = (Final weight (g) – Initial weight (g))/weeks.

Weight gain (WG; %) = $100 \times (\text{final weight (g)} - \text{initial weight (g)}) / \text{initial weight (g)}$.

Feed conversion ratio (FCR) = Total feed given (g)/Weight gain (g).

Specific growth rate (SGR; %/day) = $((\ln(\text{final weight}) - \ln(\text{initial weight})) / \text{days}) \times 100$.

Protein efficiency ratio (PER) = Weight gain (g)/Protein intake (g).

Feed conversion ratio (FCR; %) = Feed intake (g)/Weight gain (g).

Survival rate (SR; %) = $(\text{Shrimp final number} - \text{shrimp initial number}) \times 100$.

Whole body composition

Whole-body proximate composition analyses of shrimp and experimental diets were performed according to the methodology reported by AOAC (2000). Crude protein levels were determined by the Kjeldahl method, and crude lipid levels were carried out using an automated extraction method ANKOM XT-15 (Macedon, NY).

Microbial study in intestine

Using a sterile tube with 2 mL of Fix RNA (EuRx) solution, the posterior digestive contents of shrimps were suspended, and DNA extraction in posterior digestive contents was performed directly from feces with the EuRx Tissue and Bacterial DNA Purification Kit (EuRx). After measuring the quality and quantity of DNA using the SPECTROstar Nano Spectrometer, 16S Forward and 16S Reverse coded 16S Universal

bacterial primers were used to enrich the 16S rDNA V3 and V4 regions of the samples. 16sV3F (ACTCCTACGGGAGGCAGCAGT) and 16sV3R (ACCGCGGCTGCTGGCAC) primers were used for amplification of 16S rDNA genes. The primer set with the highest efficiency was selected (Çelik and Keskin 2022). PCR was performed under the following thermal cycling conditions: 35 cycles consisting of an initial warm-up step at 94 °C for 2 min, followed by denaturation at 94 °C for 1 min, acceleration at 53 °C for 45 s and elongation at 72 °C for 25 s, and finally a final elongation step at 72 °C for 5 min. The components were prepared as follows, with a total volume of 10 µL in each reaction mixture: 2 µL 5×Promega Colourless GoTaq Flexi buffer, 0.5 µL DNA template, 1.5 µL 25 mM MgCl₂, 0.8 µL 3.2 µM dNTP, 0.5 µL each primer (5 pmol/mL), 0.05 µL Taq DNA polymerase, and sterile water were added to a final volume of 10 µL. After PCR, sequencing libraries were constructed using the Nextera DNA Prep Library Preparation Kit (Illumina, San Diego, CA, USA) according to the manufacturer's recommendations, and then, it was added index codes. After that, the samples' bidirectional (2×150 bp) sequencing was performed on the Illumina iSeq 100 platform (Boyer et al. 2016). Taxonomic profiling, alignment, and identification reads were completed. Visualization, analysis, identification of OTU groups in the samples, and reporting of the data obtained were then performed using Geneious Prime and SILVAngs (Quast et al. 2012; Kaya et al. 2020).

Histological methods

Hepatopancreas samples were extracted from shrimp paralyzed in ice water, using forceps inserted beneath the carapace. The samples were placed into labelled tissue cassettes and fixed in 10% buffered formaldehyde. After 48 h, the tissue samples were processed according to a routine histology protocol. The fixative solution was initially removed following a washing step, and the tissues underwent dehydration, clearing, and paraffin infiltration procedures. Subsequently, the tissues were embedded in paraffin (Merck, paraffin beads, melting point 58 °C) to prepare paraffin blocks. Using a Shandon AES325 rotary microtome, sections of 5-µm thickness were cut from the paraffin blocks. After removing the paraffin and following hydration steps, the tissues were differentially stained with hematoxylin and eosin, and permanent slides were prepared using Entellan. The sections were examined under an Olympus CX23 trinocular microscope, and image analyses were performed using an Olympus EP50, with measurements taken and micrographs recorded.

Total hemocyte count

Hemolymph samples were collected from the shrimp's sinus using a 1-mL anticoagulant syringe with a 27-gauge needle (Ayset, Turkey). The anticoagulant solution used during the procedure was composed of 26 mM citric acid (C₆H₈O₇), 100 mM glucose, 450 mM sodium chloride (NaCl), 30 mM trisodium citrate (C₆H₅Na₃O₇), and 10 mM EDTA (C₁₀H₁₆N₂O₈), adjusted to a pH of 4.6. To determine the total hemocyte count, samples were examined under a microscope with a Neubauer counting chamber, following the method described by Söderhäll and Smith (1983).

Statistical analysis

The SPSS 17 (Chicago, IL, USA) statistical package program were used for analyses. Assumptions were tested, and analyses (ANOVA, Tukey) were performed. Firstly, the groups were examined regarding normality assumptions (the skewness and kurtosis statistics), and the homogeneity of variances was evaluated (the Levene test). The Tukey test, a post hoc analysis, was employed to identify significant differences between groups. Statistical significance was considered $P < 0.05$, and data were presented as mean \pm standard deviation.

Results

At the end of the 84 days of the trial, no significant differences existed among the seven systems in any water parameter except nitrate (Supplementary File, Table S2). Mean nitrate was significantly higher in the control than in other groups ($P < 0.05$).

The growth performance of shrimp-fed diets supplemented with *A. platensis*, probiotic mixture, and MOS levels is shown in Table 1. P, A, and S had an intermediate effect on FBW compared to the control group, while FBW improved in the ES1, ES2, and ES3 groups ($P < 0.05$). The maximum WG was achieved in the ES2 and ES3 groups, which differed from the C group ($P < 0.05$). PER values showed a significant increase in all experimental groups compared to the control group ($P < 0.05$), and the maximum values were reached in the synbiotic groups. Similarly, FCR levels improved in the experimental groups and the best values were obtained from the synbiotic groups ($P < 0.05$). No significant difference was observed among treatments for survival in all groups.

Regarding the effects of different feed additives, no significant difference was determined in whole-body protein content among the treatments (Table 2). However, there was a significant increase in the crude lipid of shrimp in the S, ES1, and ES2 groups compared to the control group ($P < 0.05$). Ash in shrimp fed the ES1 diet was higher than in shrimp fed the control diet.

At the end of the trial, it was determined that dietary supplementation of feed additives changed the gut microbiota composition in brown shrimp (Figs. 1 and 2). Bacillota (42.76%), Bacteroidota (22.60%), and Proteobacteria (16.18%) were the most dominant phyla in the C group. Proteobacteria was recorded as the most dominant phylum in all groups except the C group. The highest abundance of Proteobacteria was observed in A group (62.67%), followed by P (58.33%), ES3 (45.87%), ES1 (39.78%), ES2 (39.24%), and S (35.40%) groups. Bacillota was the dominant phylum in the C group, while its abundance ranged from 1.98 to 13.47% in the experimental groups. Bacteroidota had high abundance in all groups, with the highest rate observed in the ES1 group (29.92%), and relatively high rates of this phylum were also observed in the P (24.68%), C (22.60%), and ES3 (21.62%) groups. The phylum Actinomycetota was found above 1% in all groups, with the highest abundance observed in the S group at 5.36%. In the other groups, the abundance ranged between 1.24 and 2.28%. Verrucomicrobiota abundance was generally low, with the highest abundance percentage found in the C group (8.82%) and lower levels in the other groups, ranging from 1.05 to 4.09%. In addition to phylum-level changes, genus-level differences also showed the effect of dietary probiotics, prebiotics, and synbiotics on bacterial composition (Fig. 3). The C group exhibited a more heterogeneous microbial composition,

Table 1 Growth parameters of brown shrimp grown in tanks with different feed additive groups for 84 days

Parameters	C	P	A	S	ES1	ES2	ES3
IBW	1.21 ± 0.01	1.21 ± 0.01	1.21 ± 0.01	1.21 ± 0.01	1.21 ± 0.00	1.21 ± 0.00	1.21 ± 0.00
FBW	7.86 ± 0.95a	8.12 ± 0.58ab	8.40 ± 0.90abc	8.45 ± 0.63abc	8.80 ± 0.60bc	9.01 ± 0.72c	8.95 ± 0.64c
WG	6.65 ± 0.18a	6.90 ± 0.20ab	7.19 ± 0.17bc	7.24 ± 0.14bc	7.58 ± 0.14 cd	7.80 ± 0.04d	7.74 ± 0.06d
DWG	0.08 ± 0.01a	0.08 ± 0.00a	0.08 ± 0.00ab	0.08 ± 0.00abc	0.08 ± 0.00bc	0.09 ± 0.00c	0.09 ± 0.00bc
WWG	0.53 ± 0.04a	0.54 ± 0.02a	0.56 ± 0.01ab	0.56 ± 0.01abc	0.59 ± 0.01bc	0.61 ± 0.00c	0.60 ± 0.00bc
WGR	548.88 ± 15.12a	569.35 ± 15.93ab	593.12 ± 14.35bc	597.27 ± 12.45bc	625.51 ± 11.58 cd	643.37 ± 3.56d	638.33 ± 5.44d
SGR	2.13 ± 0.11a	2.11 ± 0.03a	2.15 ± 0.02a	2.16 ± 0.02a	2.20 ± 0.02a	2.23 ± 0.01a	2.22 ± 0.01a
PER	1.15 ± 0.03a	1.29 ± 0.04b	1.47 ± 0.04c	1.64 ± 0.03d	1.71 ± 0.03de	1.76 ± 0.01e	1.75 ± 0.01e
FCR	2.29 ± 0.06c	2.21 ± 0.06 cd	2.12 ± 0.05bc	2.11 ± 0.04bc	2.01 ± 0.04ab	1.96 ± 0.01a	1.97 ± 0.02a
Survival (%)	86.67 ± 2.89	86.67 ± 2.89	88.33 ± 2.89	86.67 ± 2.89	88.33 ± 2.89	88.33 ± 2.89	86.67 ± 2.89

The data correspond to the mean ± standard deviation. Different lowercase letters in each line demonstrated significant differences ($P < 0.05$). C: control group with no feed additive, P: probiotic mixture (1 mL, 1×10^9 CFU/100 g feed, *Lactobacillus plantarum*, *L. acidophilus*, *L. salivarius*, and *Bacillus subtilis*), A: 0.2 g Arthrospira/100 g feed, S: probiotic mixture (1 mL, 1×10^9 CFU/100 g feed)+0.2 g Arthrospira/100 g feed, ES1: probiotic mixture (1 mL, 1×10^9 CFU/100 g feed)+0.2 g Arthrospira/100 g feed+0.1 g MOS/100 g feed, ES2: probiotic mixture (1 mL, 1×10^9 CFU/100 g feed)+0.2 g Arthrospira/100 g feed+0.2 g MOS/100 g feed, ES3: probiotic mixture (1 mL, 1×10^9 CFU/100 g feed)+0.2 g Arthrospira/100 g feed+0.3 g MOS/100 g feed

Abbreviations: IBW initial body weight (g), FBW final body weight (g), WG weight gain, DWG daily weight gain, WWG weekly weight gain, WGR weight gain rate, SGR specific growth rate, PER protein efficiency ratio, FCR feed conversion ratio

Table 2 Whole body composition of brown shrimp grown in tanks with different feed additive groups for 84 days

Parameters (%)	C	P	A	S	ES1	ES2	ES3
Crude protein	15.94±0.15	16.03±0.23	16.10±0.10	16.09±0.25	16.19±0.06	16.24±0.21	16.15±0.15
Crude lipid	2.27±0.04 ^a	2.28±0.03 ^a	2.32±0.04 ^{ab}	2.34±0.03 ^b	2.36±0.02 ^b	2.35±0.02 ^b	2.32±0.02 ^{ab}
Ash	3.17±0.08 ^a	3.19±0.03 ^a	3.19±0.05 ^a	3.25±0.06 ^{ab}	3.30±0.04 ^b	3.24±0.06 ^{ab}	3.26±0.02 ^{ab}

C: control group with no feed additive, P: probiotic mixture (1 mL, 1×10⁹ CFU/100 g feed, *Lactobacillus plantarum*, *L. acidophilus*, *L. salivarius*, and *Bacillus subtilis*), A: 0.2 g Arthrospira/100 g feed, S: probiotic mixture (1 mL, 1×10⁹ CFU/100 g feed)+0.2 g Arthrospira/100 g feed, ES1: probiotic mixture (1 mL, 1×10⁹ CFU/100 g feed)+0.2 g Arthrospira/100 g feed+0.1 g MOS/100 g feed, ES2: probiotic mixture (1 mL, 1×10⁹ CFU/100 g feed)+0.2 g Arthrospira/100 g feed+0.2 g MOS/100 g feed, ES3: probiotic mixture (1 mL, 1×10⁹ CFU/100 g feed)+0.2 g Arthrospira/100 g feed+0.3 g MOS/100 g feed

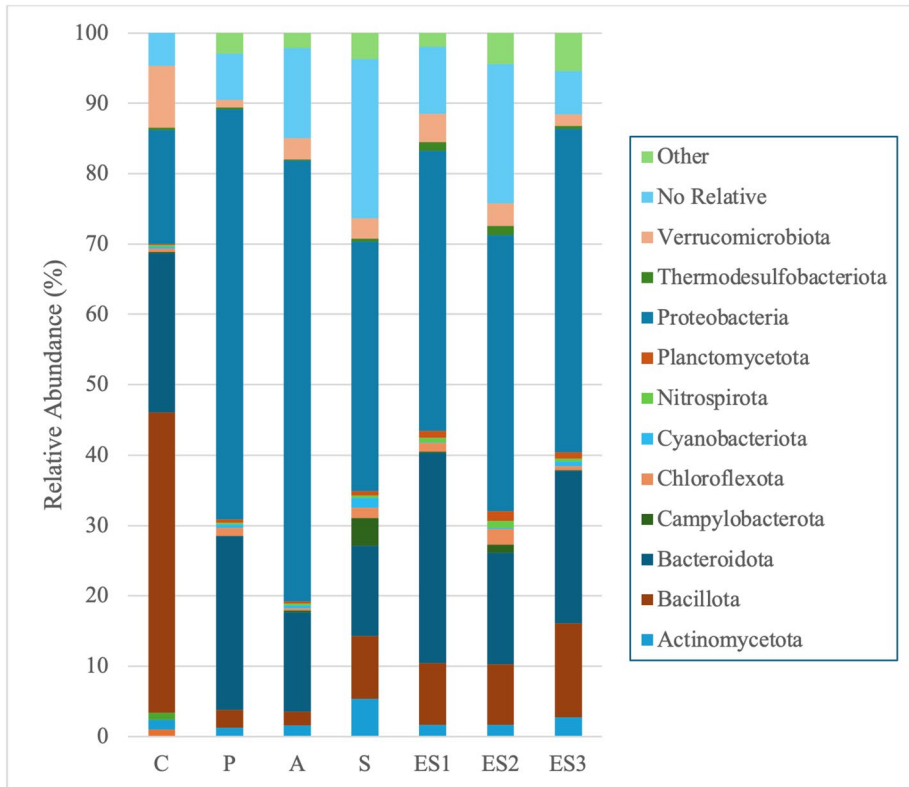
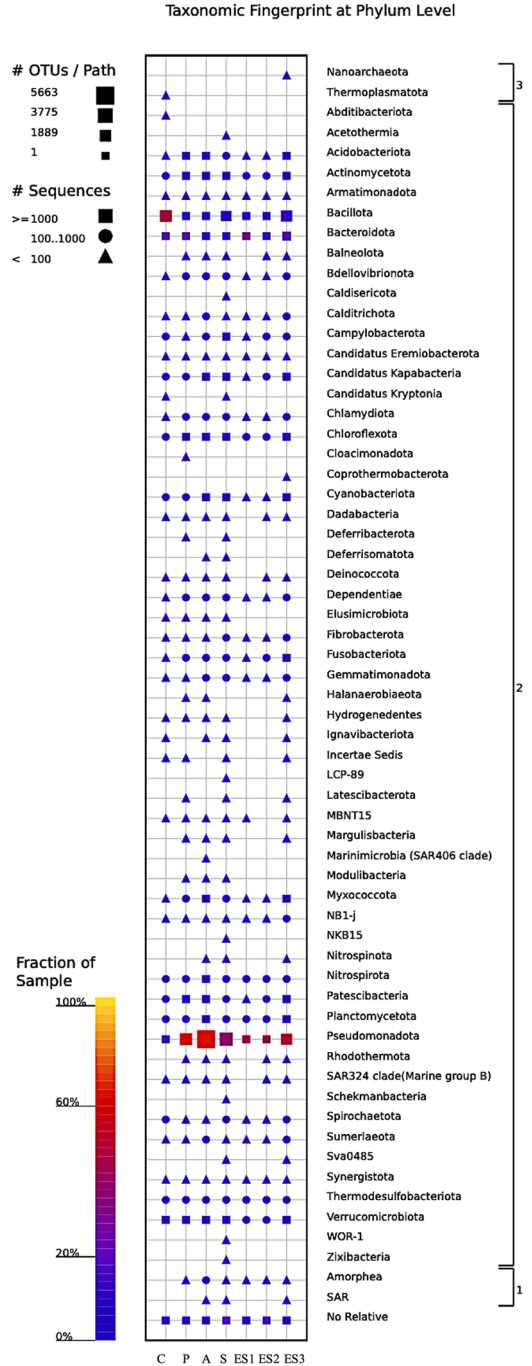


Fig. 1 Phylum level taxonomic composition of the intestinal microbiome in shrimp fed different diets (top 10 of total sequences)

Fig. 2 Phylum level proportional abundance of microbiota composition in shrimp fed different diets



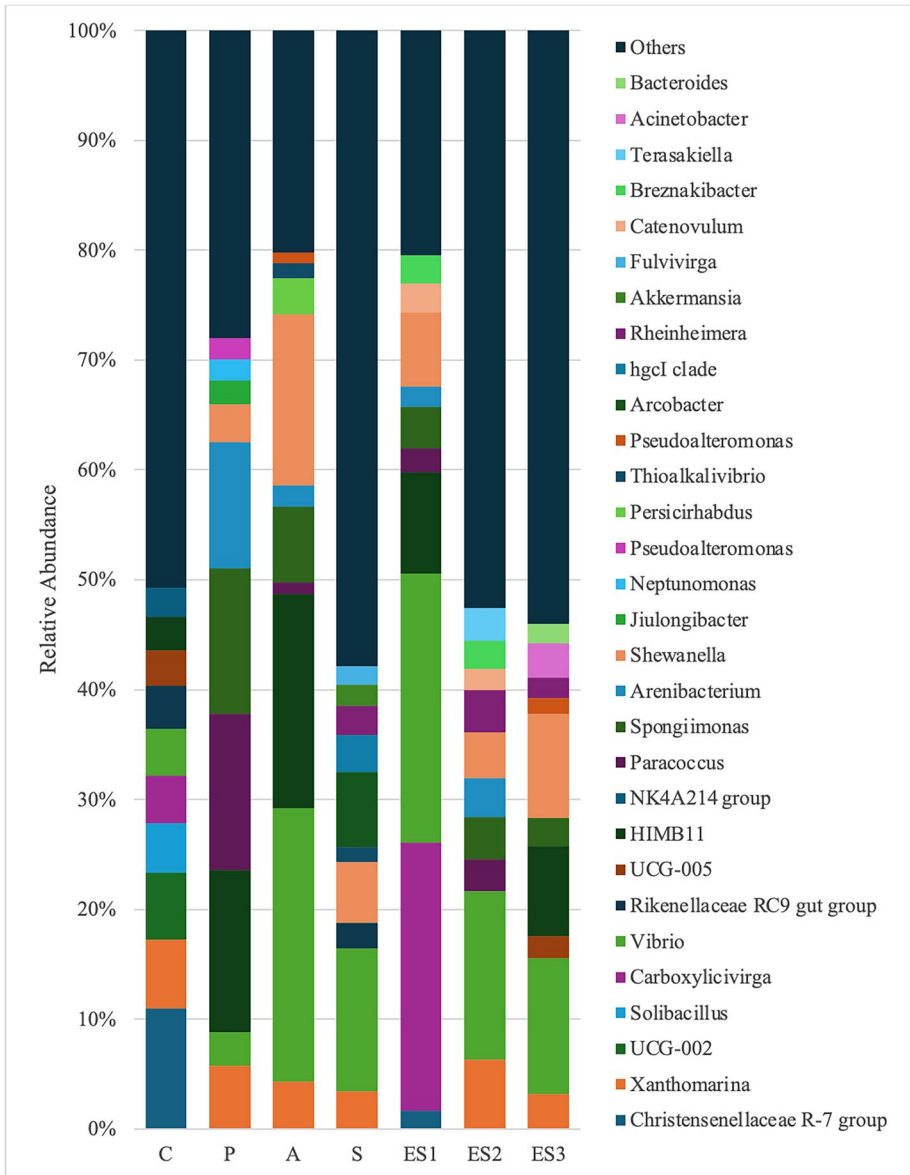


Fig. 3 Genus level taxonomic composition of microbiota composition in shrimp fed different diets

while experimental groups led to notable shifts in specific bacterial genera. In particular, the relative abundance of *Vibrio* increased in A, S, ES1, ES2, and ES3 groups.

The alpha diversity indices showed that the control group had higher values than the experimental groups for the Shannon index, but the experimental groups reached higher values for the Simpson indices (Fig. 4).

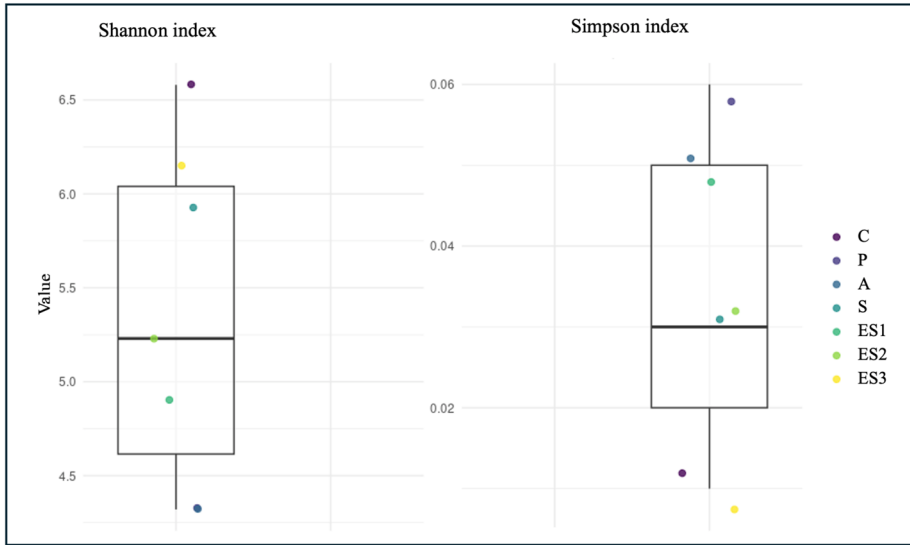


Fig. 4 Diversity indexes of intestinal microbiota in shrimp fed different diets

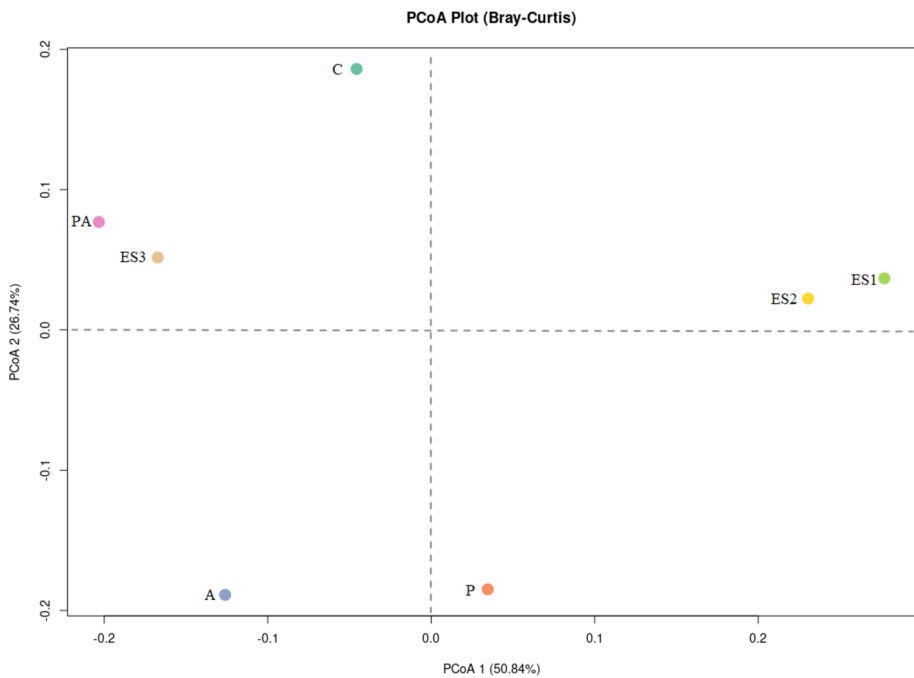


Fig. 5 The principal coordinate analysis (PCoA) of bacterial community of intestinal microbiota in shrimp fed different diets

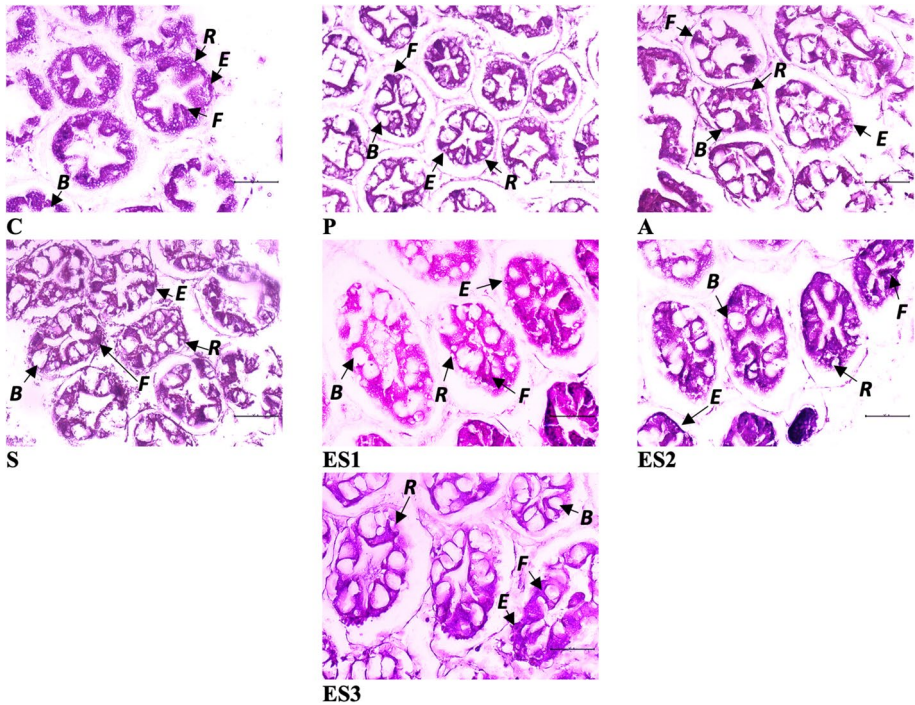


Fig. 6 Sections of the hepatopancreas representing the groups after the feeding trial in brown shrimp. In shrimp fed with diets supplemented with additives (probiotics, prebiotics, symbiotics), an increase in the ratio of vacuoles in the hepatopancreatic tubular epithelium, and consequently in B cells, was observed compared to shrimp in the control group fed with the basal experimental diet. The other cell types (F, E, R cells) of hepatopancreas sections were observed to show a similar distribution across all experimental groups, C: control group with no feed additive, P: probiotic mixture (1 mL, 1×10^9 CFU/100 g feed, *Lactobacillus plantarum*, *L. acidophilus*, *L. salivarius*, and *Bacillus subtilis*), A: 0.2 g *Arthrospira*/100 g feed, PA: probiotic mixture (1 mL, 1×10^9 CFU/100 g feed)+0.2 g *Arthrospira*/100 g feed, S1: probiotic mixture (1 mL, 1×10^9 CFU/100 g feed)+0.2 g *Arthrospira*/100 g feed+0.1 g MOS/100 g feed, S2: probiotic mixture (1 mL, 1×10^9 CFU/100 g feed)+0.2 g *Arthrospira*/100 g feed+0.2 g MOS/100 g feed, S3: probiotic mixture (1 mL, 1×10^9 CFU/100 g feed)+0.2 g *Arthrospira*/100 g feed+0.3 g MOS/100 g feed, H&E, bar: 50 μ m

The principal coordinate analysis (PCoA) analysis based on Bray–Curtis distances was used to assess differences in bacterial community structure (Fig. 5). The results showed that different additives lead to various compositions in the gut microbiota of shrimp.

Figure 6 shows the histological structure of the hepatopancreas in brown shrimp fed with diets enriched with probiotics, prebiotics, and synbiotic. Four different cell types were identified in the hepatopancreas tubules in the hematoxylin and eosin-stained paraffin-embedded sections examined under a light microscope. These are blaszellen (B cells), fibrillenzellen (F cells), restzellen (R cells), and embryonalzellen (E cells), which are marked on the micrographs. It was noted that the number of B cells increased in the hepatopancreas of brown shrimp fed with diets containing feed additives, indicating a significant effect in the hepatopancreas tubules. In the control group, the number of B cells ranged from 4.60 ± 0.89 per tubule, while in shrimp fed with P-supplemented diets, the counts were 5.40 ± 0.55 ; in shrimp fed with A-supplemented diets, 6.40 ± 0.55 ; in the S

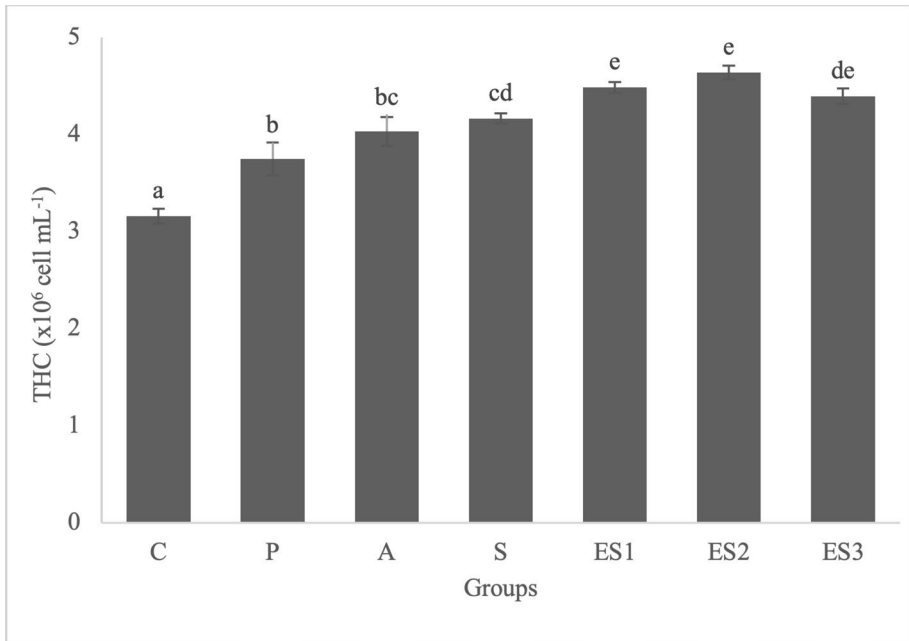


Fig. 7 Total hemocyte counts (THC) of shrimp fed different diets

group, 6.20 ± 0.45 ; in the ES1 group, 7.20 ± 1.64 ; in the ES2 group, 7.60 ± 0.55 ; and in the ES3 group, 8.20 ± 1.30 B cells per tubule were counted and recorded.

The total hemocyte counts of the shrimp fed different diets are summarized in Fig. 7. The C group had the lowest THC value and was significantly different from the trial groups ($P < 0.05$). The P group showed a significant increase compared to the control group but was statistically similar to the A group. The THC value in the S group was higher than in the C and P groups, with a statistically significant difference ($P < 0.05$). ES1 and ES2 groups showed the highest THC levels and both groups were statistically in the highest class ($P < 0.05$). The ES3 group showed a slight decrease compared to the ES1 and ES2 groups, but no statistical difference was found.

Discussion

The water quality and the feed's nutrient content are the main factors affecting production success in shrimp culture under BFT (El-Sayed 2021). BFT is a potential technique for water quality improvement, and optimized water quality enhances healthy culture parameters in shrimp (Kumar et al. 2018; Kaya et al. 2020). The present study showed that the dietary administration of probiotics, prebiotics, and synbiotic in brown shrimp culture did not affect the water quality parameters of the BFT environment except for nitrate, and all water quality values were within acceptable ranges for BFT in shrimp culture (Avnimelech 2012).

The present study showed that growth performance was improved in brown shrimp (*Penaeus aztecus*) fed synbiotic (probiotic mixture + *Arthrospira*: S) and enriched synbiotic (probiotic mixture + *Arthrospira* + MOS: ES1, ES2, and ES3) diets. Although previous

studies have investigated the use of probiotics, *Arthrospira* and MOS, this is the first study to examine their effects in combination with BFT conditions. Genc et al. (2024b) reported that adding 3 g kg⁻¹ MOS to the diet could significantly improve WG and SGR for brown shrimp (*Penaeus aztecus*) grown in the BFT system. However, Rodrigues et al. (2018) found that dietary mannoprotein (0.02, 0.08, and 1.2%) as a prebiotic did not affect the growth performance of Pacific white shrimp (*Penaeus vannamei*) under BFT conditions. To our knowledge, no study has investigated the effects of dietary supplementation of *Arthrospira* on aquatic organisms under BFT conditions. He et al. (2023) reported that probiotic addition to water or aquafeed did not change the growth performance of Pacific white shrimp under BFT conditions. Synbiotic, a combination of probiotics and prebiotics with synergistic benefits on the host, have been used in aquafeeds for many years (Khanjani et al. 2024). These additives have become one of the focal points of studies with their growth-promoting effects in shrimp culture (Huynh et al. 2018). Dietary synbiotic supplementation with *Lactobacillus plantarum* and pectin improved growth and feed efficiency in Pacific white shrimp, while *L. plantarum* or pectin alone did not affect growth performance (Kuo et al. 2021). Furthermore, the synbiotic supplementation of *Leuconostoc mesenteroide* (strain B4) and dextran increased growth in Pacific white shrimp (Huang et al. 2023), and the combination of moringa (*Moringa oleifera*) and *L. acidophilus* provided the highest growth performance in Pacific white shrimp (Das et al. 2024). In this study, using synbiotic, as in previous studies mentioned above, improved growth performance of brown shrimp in BFT. The improved growth indices in synbiotic groups may be due to increased digestion and absorption of the diet caused by synbiotic. This observation aligns with Anguiano et al. (2013), who demonstrated that the presence of beneficial bacteria in the host's gut can improve nutrient absorption by optimizing the gut environment. This improvement in nutrient absorption may contribute to better overall health and growth in the host. Additionally, the diversity of the gut microbiome occurring in synbiotic groups may have shown advantageous effects on growth.

The gut microbiome plays a crucial role in modulating gut metabolism, enhancing nutrient absorption, and regulating the immune response, as highlighted by Goh et al. (2023). This complex microbial community not only aids in the efficient processing and uptake of nutrients but also contributes to maintaining gut health by supporting immunity. In this context, examining gut microbiota is probably a key point for understanding the growth-promoting effect of probiotics, prebiotics, and synbiotic. The present study identified Proteobacteria as the most dominant phylum in all experimental groups. In the present study, Proteobacteria emerged as the most dominant phylum in all experimental groups. Interestingly, its dominance was significantly higher in groups P and A, while a decrease was observed in the synbiotic groups, leading to a more balanced microbial composition. This change suggests that synbiotic intervention may help regulate Proteobacteria levels, potentially promoting a healthier and more stable gut microbiota composition. This study is consistent with previous findings on the influence of probiotics and synbiotic on the abundance of Proteobacteria in the gut microbiota of aquatic species. Similar to the present study results, elevated levels of Proteobacteria were observed in the gut microbiota of Pacific white shrimp following dietary supplementation with probiotics and synbiotic (Hasyimi et al. 2020). Du et al. (2022) similarly reported increased Proteobacteria in Pacific white shrimp after administering *L. plantarum*. Correspondingly, Jaramillo-Torres et al. (2019) noted a rise in Proteobacteria in salmon (*Salmo salar*) treated with *Pediococcus acidilactici*, and Xia et al. (2020) observed comparable increases in tilapia (*Oreochromis niloticus*) fed with *Bacillus subtilis* and *B. cereus*. Notably, while an abundance of Proteobacteria in shrimp has often been linked to general health status, Pardede et al. (2024) caution that it may also indicate a potential disease

risk. Conversely, Lee et al. (2022) demonstrated that supplementation with *B. subtilis* and lactic acid bacteria in the Pacific white shrimp diet increased Proteobacteria levels and enhanced resistance to *Vibrio* infection. These findings suggest that Proteobacteria abundance's cultured species health impact may vary based on specific probiotic strains and environmental factors, potentially providing resilience in certain contexts. In the present study, following the Proteobacteria phylum, the Bacteroidota phylum was the second most dominant in the gut microbiota of brown shrimp. This phylum has been linked to enhanced growth, fermentation, and metabolic functions in shrimp (Hasyimi et al. 2020). Previous research has consistently reported that the gut microbiota of various aquatic organisms, including shrimp, is typically dominated by Proteobacteria and Bacteroidota (Rungrassamee et al. 2014; Zheng et al. 2017; Diwan et al. 2022). These two phyla, regarded as stable members of the shrimp gut microbiota, are often found to dominate across all growth stages (Li et al. 2018). It is worth mentioning that Bacillota (Firmicutes), the most abundant phylum in the C group, was present at lower levels in the experimental groups, particularly in the P and A groups. This reduction suggests that dietary supplementation with prebiotics, probiotics, and synbiotic under BFT conditions influenced the gut microbiota composition of brown shrimp, contributing to shifts in the microbiota balance.

The Shannon index data indicated that the C and ES3 groups had the highest diversity values, suggesting that a diet without additives or with a specific combination of additives may positively influence the microbial diversity in the shrimp gut under BFT. Conversely, lower Shannon index values were recorded in the P group, where a probiotic mixture was added, indicating that probiotics may have a limiting effect on intestinal microbial diversity. This aligns with the findings of Luo et al. (2024), who reported that adding probiotics to the diet of Pacific white shrimp significantly decreased the Shannon indices, reducing bacterial diversity compared to the control. Simpson index results further supported these observations, showing the lowest values in the C and ES3 groups, which displayed higher microbial diversity and density than the other groups. The high microbial diversity in these groups suggests that certain additive combinations may effectively preserve or enhance intestinal microbial balance. These findings emphasized the importance of considering microbial diversity when selecting dietary additives to optimize shrimp intestinal health.

The hepatopancreas (HP) is a vital digestive organ of decapods that carries out functions similar to the liver, pancreas, intestines, and other digestive organs in vertebrates. It has been the focus of scientific research for over a century, particularly for understanding nutritional phenomena and assessing health conditions through histological examinations. In this context, it has also been studied as a tissue indicator of health. Some studies associate the proper functioning of the hepatopancreas with improved farming productivity and survival rates (Kanazawa 1985; Genc et al. 2007, 2024a, b; Zhu et al. 2019; Pourmzaffar et al. 2019; Kaya et al. 2019, 2020; Wei et al. 2023; Nguyen 2024). In general, because it is a significant indicator of shrimp health, it can be used to confirm feeding data. The tubular epithelium of the hepatopancreas is an important indicator of the synthesis and secretion of digestive enzymes into the lumen and, therefore, of food digestion and nutrient absorption. The hepatopancreas also plays a role in the storage of inorganic substances and in lipid and carbohydrate metabolism. It acts as the primary metabolic center during molting. The small vacuolated cells, known as restzellen or R cells, identifiable in histological sections of the tubule, trap unwanted inorganic substances such as heavy metals, contributing to the detoxification process. The hepatopancreas tubules are known to be composed of five main cell types: restzellen (R cells), which are absorptive and storage cells; embryonalzellen (E cells); fibrillenzellen (F cells), responsible for the synthesis of digestive enzymes and hemocyanin; blasenzellen (B cells), which have large vacuoles and are involved in

intracellular digestion and the secretion of digestive enzymes; and midgut cells (M cells), which are thought to function in storage and cell renewal. However, it is noted that B, F, R, and E cells are dominant in the hepatopancreas (Al-Mohanna and Nott 1986; Genc et al. 2007; Li et al. 2008; Vogt 2019, 2020). The sections showing the hepatopancreas histology of brown shrimp showed that the tubular epithelial architecture displayed normal morphology across all groups. As previous studies have suggested that hepatopancreas morphology is an indicator of health, the brown shrimp represented in the examined sections were evaluated as healthy. Among the five cell types typically found, four dominant types were identified, with B cells characterized by large and prominent vacuoles, being the most noticeable. It was also determined that the number of B cells increased in groups with dietary additives. The increase in B cells, responsible for the production and secretion of digestive enzymes, was interpreted as an indicator of successful digestion and nutrient absorption. This finding is consistent with the literature (Al-Mohanna and Nott 1986, Genc et al. 2007, Zhu et al. 2019, Kaya et al. 2019; 2020 Vogt 2019; 2020, Wei et al. 2023, Genc et al. 2024a, b, Nguyen 2024). Li et al. (2008) reported that in Pacific white shrimp cultured at three different salinities, the number of B cells increased at salinity levels of 3 ppt and 32 ppt, along with an increase in their area and thus volume. However, at a salinity of 17 ppt, the number of B cells decreased. This finding aligns with the present study results, where we observed an increase in B cells in BFT environment at a similar salinity level to the optimal salinity identified in the previous study, particularly with feed additives.

In crustaceans, an increased total hemocyte count (THC) is a key indicator of immune response, as hemocytes regulate immune activities like phagocytosis and encapsulation to defend against infection (Panigrahi et al. 2020; Yu et al. 2024). It is suggested that the immune system of crustaceans, including shrimp, can recognize foreign particles such as probiotics, which in turn strengthens immune defenses (Pardede et al. 2024). Beyond probiotics, dietary synbiotic may further enhance this immune response. Several studies have reported that dietary synbiotic improve immune parameters in shrimp. For instance, THC levels in Pacific white shrimp fed a synbiotic combination of pectin and *L. plantarum* were significantly elevated (Kuo et al. 2021). Similarly, a 30-day synbiotic treatment with multiple species increased THC in Pacific white shrimp (Utomo et al. 2023). In another study by Pardede et al. (2024), Pacific white shrimp receiving dietary synbiotic showed higher THC levels compared to the control group. Consistent with these findings, the current study demonstrated that dietary synbiotic elevated THC levels in shrimp, supporting the notion that synbiotic can improve immune responses.

Conclusion

In the present study, the administration of dietary synbiotic to brown shrimp under BFT conditions supported growth performance and modulated gut microbiota diversity, potentially fostering a more balanced and resilient intestinal environment. As a result, observed improvements in growth, microbiome composition, THC levels, and hepatopancreas histomorphology indicate that dietary synbiotic hold significant value in optimizing brown shrimp diets and support their recommendation to enhance productivity in BFT systems. Given its strong growth potential and high survival rate under BFT conditions, brown shrimp is considered a viable alternative to Pacific white shrimp. Further research is needed to better understand the mechanisms underlying the growth-promoting effects of dietary synbiotic in brown shrimp.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding authors upon reasonable request.

Declarations

Competing interests The authors declare no competing interests.

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