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Effect of biofloc technology in *Farfantepenaeus aztecus* culture: The optimization of dietary protein level on growth performance, digestive enzyme activity, non-specific immune response, and intestinal microbiota

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Abstract

In this study, a biofloc technology (BFT) based 90-day feeding trial was performed to estimate the effects of four different dietary protein levels (290, 320, 350 and 380 g protein kg^{-1} diet; 29P, 32P, 35P, and 38P, respectively) on growth performance, digestive enzyme activity, non-specific immune response, whole-body proximate and fatty acid composition and intestinal microbiota of *Farfantepenaeus aztecus*. Final weight, weight gain, and specific growth rate improved with protein levels up to 35P per kg of diet, below which decreased significantly. Significant differences were observed in total hemocyte count, acid phosphatase, respiratory burst, glutathione, alkaline phosphatase, and total antioxidant capacity indices of shrimp fed different dietary protein levels. Protease, lipase, and amylase activities of shrimp were also affected by dietary protein levels. Proteobacteria was the most abundant bacteria among the groups. Overall, this study revealed that using 35% dietary protein is optimal in

F. aztecus culture under environmentally friendly BFT conditions. Furthermore, these results provide a theoretical basis for future research on the evaluation of functional ingredients in diet for more economical production of *F. aztecus* at optimal protein levels.

KEYWORDS
dietary protein, digestive enzyme activity, immune response, microbiota, shrimp culture

1 | INTRODUCTION

The consumption capacity of diversified and nutritious foods has increased with the significant contributions made to aquaculture production in the last two decades (Ahmed & Ahmad, 2020). The demand for aquatic products for human consumption has dramatically enhanced as a result of the increasing population and public awareness (FAO, 2020). To meet the increasing demand, nutritionally balanced ingredients for the species must be formulated at the lowest cost, ensuring optimal growth performance (Jia et al., 2022). Protein is the primary ingredient of the diet for growth in cultured species. Dietary protein directly affects healthy growth, feed intake, and feed costs in aquaculture and is the most expensive nutrient in the diet (Sivaramakrishnan et al., 2022). Fishmeal is the primary source of protein in aquaculture feed and has high percentages in aquaculture formulations (Wang et al., 2022). This is as a result of its high protein content, balanced amino acid composition, and fatty acid profile (Tacon & Metian, 2015). However, reducing the amount of fishmeal in the diet may cause changes in gut microbiota (J. X. Liu et al., 2022; Yang et al., 2022) and digestive enzyme activity, antioxidant activity, and immune response of cultured species may also be negatively affected, resulting in reduced growth performance (Chaklader et al., 2020). In this context, exploring new approaches to support healthy growth is vital to minimize the potential impacts of increasing fishmeal shortages in aquaculture. Biofloc technology (BFT) is an innovative technique in aquaculture that maintains water quality with minimal or no water changes, increases growth performance, and improves the feed conversion ratio (Avnimelech, 2015; Mansour et al., 2022). Biofloc in the rearing water is consumed by cultured species as extra food and can replace a considerable part of dietary protein (Sharawy et al., 2022; Yun et al., 2016). Khanjani (2015) concluded that approximately 30% of the daily nutrient intake in *Litopenaeus vannamei* could be supplied by biofloc. Megahed and Mohamed (2014) also stated that dietary protein levels could be reduced from 45% to 25% in the BFT without affecting growth in *Fenneropenaeus indicus* culture. Through the biochemical composition of biofloc, essential nutrients such as protein can be provided for the cultured species (Khanjani & Sharifinia, 2020).

Penaeid shrimps are the most valuable resource among crustaceans in the aquaculture industry (FAO, 2020; Kaya et al., 2020). Brown shrimp, *Farfantepenaeus aztecus* (also known as *Penaeus aztecus*) of western Atlantic origin, is regarded as one of the most important commercial species in the Atlantic Coast United States and the Gulf of Mexico (Al-Badran et al., 2019). The first record of brown shrimp in the Mediterranean was reported in 2009 from the Antalya coast (Deval et al., 2010) and then from the coastal regions from Finike to Iskenderun (Eastern Mediterranean coast, Turkey) (Gökoğlu & Özvarol, 2013; Stergiou et al., 2014). Evaluation of culture conditions and optimization of dietary protein requirement in BFT of brown shrimp is unknown and this knowledge is necessary to develop a species-specific nutritionally balanced diet (Chen et al., 2023; Klett & Watson, 2020). For this reason, we aimed to reveal the aquaculture performance of this species, which can be an alternative/candidate for crustacean culture. In this study, the effects of feeding brown shrimp juveniles feed with different protein levels in the BFT system on growth performance, digestive enzyme activity, non-specific immune response, and gut microbiota profile were evaluated for the first time.

2 | MATERIALS AND METHODS

2.1 | Study location and shrimp supply

The experiment was carried out at the Fisheries Research and Application Unit, Faculty of Agriculture, Ankara University, Ankara, Turkey. In the study, juvenile brown shrimp was used. Production of shrimps was performed at the Research Unit, Faculty of Marine Sciences, Iskenderun Technical University, Hatay, Turkey. After the spawning of brown shrimp broodstock, the incubation period and the hatching of eggs were accomplished. Until the juvenile stage, feeding of larvae and postlarvae was applied with algae, Artemia, and micro diet. Juvenile shrimps (<1 g) were transported to the Fisheries Research and Application Unit in a plastic tank (200 L) under aeration conditions 15 days before the experiment.

2.2 | Feed formulation and preparation of experimental diets

The main protein sources were fish meal, soy protein concentrate, soybean meal, wheat gluten, corn gluten, and pea protein. Fish oil and soybean oil were used as the primary lipid sources. The protein level of the prepared shrimp diet is within the range recommended for shrimp by the National Research Council (2011). Four experimental diets were formulated to contain different levels (38%, 35%, 32%, and 29%) of dietary protein with a lipid level of 10.4%. The ingredients and nutritional composition of the diets are listed in Table 1. Dry mixing ingredients with fish oil manufactured diets

TABLE 1 Formulation and proximate composition of experimental diets.

Ingredients	P38	P35	P32	P29
Fish meal	30	24.2	19	14.2
Soy protein concentrate	4	4	4	4
Soybean meal	8	8	8	8
Wheat gluten	1	1	1	1
Corn gluten	12	12	12	12
Pea protein concentrate	2	2	2	2
Wheat flour	27.8	33.4	38.3	42.7
Wheat middlings	5	5	5	5
Fish oil:soy oil (1:1)	6	6.2	6.5	6.9
Vitamins premix	0.5	0.5	0.5	0.5
Minerals premix	0.2	0.2	0.2	0.2
Vitamin C	0.1	0.1	0.1	0.1
Guar gum	3	3	3	3
Cholesterol	0.4	0.4	0.4	0.4
Total (∼)	100	100	100	100
Proximate composition (%)[a]				
Crude protein	37.94 ± 0.26	35.09 ± 0.31	32.24 ± 0.28	28.76 ± 0.39
Crude fat	10.33 ± 0.64	10.37 ± 0.48	10.42 ± 0.60	10.44 ± 0.44
Ash	7.76 ± 0.19	7.42 ± 0.18	7.36 ± 0.44	7.33 ± 0.27
Crude cellulose	1.87 ± 0.24	1.99 ± 0.19	2.14 ± 0.33	2.3 ± 0.17
Moisture	11.18 ± 0.53	11.12 ± 0.48	11.17 ± 0.44	11.18 ± 0.48

[a]Results are given as mean (±SD).

TABLE 2 The profiles of amino acids in the experimental diets (mg g^{-1}).

Amino acids	38P	35P	32P	29P
Methionine	0.79	0.76	0.75	0.73
Cystine	0.49	0.47	0.46	0.42
Lysine	2.23	2.21	2.20	2.17
Tryptophan	0.28	0.26	0.26	0.21
Threonine	1.12	1.10	1.09	1.07
Isoleucine	1.41	1.40	1.40	1.40
Histidine	0.97	0.95	0.95	0.94
Valine	1.56	1.54	1.52	1.51
Leucine	3.04	3.02	3.01	3.00
Arginine	1.73	1.72	1.72	1.70
Phenylalanine	2.42	2.41	2.40	2.38

before utilizing a laboratory food mixer. The mixing was continued until a homogenous blend was obtained. The resulting paste was extruded through a cold press extruder (PTM P6 Extruder System) using a 1 mm aperture die. All diets were subsequently dried in a dehumidifying oven for 24 h at 40°C and then stored in plastic bags at −18°C until required.

The profiles of amino acids and fatty acids in the diets are detailed in Tables 2 and 3.

2.3 | Experimental setup and culture conditions

A 90-day experiment was conducted in four indoor recirculating aquaculture systems (RAS) with 3 fiberglass tanks each (45 L, 20 cm water depth) with a bottom area of 0.24 m^2 and 4 L min^{-1} water flow per tank. The RAS was set up before the experiment (34 ± 1 ppt, 28 ± 1°C) using artificial seawater (Caledonia Reef Salt, ReeFlowers, Istanbul, Turkey) with produced biofloc with an optimal C:N ratio of 15:1 (Avnimelech, 2012). The C:N ratio of the feed utilized was 8.19, and daily additions of 601.4 g sugar beet molasses as a commonly available carbon source (procured from Ankara Sugar Company, Turkey, 50% carbon content) were made per 1 kg feed to achieve a C:N ratio of 15:1. The experiment commenced once the biofloc volume reached 10 mL L^{-1}. To maintain the ideal C:N ratio, molasses was added throughout the trial after feeding. During the trial period, the biofloc volume was measured daily using the Imhoff cone.

The shrimp were acclimated (15 min, 1 ppt) in a fiberglass tank (250 L water volume) filled with aerated artificial seawater (34 ± 1 g L^{-1} and 28 ± 1°C) for 15 days before the beginning of the experiment. The shrimp were fed thrice daily with the control diet (38% CP) during the acclimation period.

A total of 240 healthy juveniles of *F. aztecus* (weighing 0.840 ± 0.41 g) were randomly stocked in 12 experimental units, each containing 20 shrimp (83 shrimps m^{-2}). The 12 experimental units were divided into four treatments with three replicates. In the experiment, 12 h of light and 12 h of dark photoperiod were applied using a timer, and feeding was performed three times daily at 08.00, 13.00, and 18.00 h. Feeding was started at 6% of body weight and gradually reduced by 0.6% every 15 days to 3%. A refractometer was utilized to monitor the optimal salinity daily (34 ppt). In addition, high-quality freshwater (obtained via a reverse osmosis system) was used in the RAS to balance out any salinity increase as a result of evaporation losses.

2.4 | Water quality

During the experiment, water temperature, salinity, pH, and dissolved oxygen values (YSI® 556, YSI Inc., Yellow Springs, OH, USA) were measured daily to maintain optimum levels. Daily follow-up was performed using an

TABLE 3 The profiles of fatty acids in the experimental diets (mg g^{-1}).

Fatty acids	38P	35P	32P	29P
12:0	0.10 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.11 ± 0.02
13:0	0.18 ± 0.01	0.15 ± 0.01	0.10 ± 0.01	0.16 ± 0.02
14:0	1.70 ± 0.03	1.51 ± 0.06	1.58 ± 0.02	1.57 ± 0.02
15:0	0.78 ± 0.02	0.57 ± 0.02	0.65 ± 0.05	0.64 ± 0.01
16:0	13.03 ± 0.45	11.70 ± 0.46	11.30 ± 1.01	13.19 ± 0.12
17:0	1.15 ± 0.14	1.24 ± 0.12	1.08 ± 0.14	1.13 ± 0.13
18:0	8.38 ± 0.08	8.09 ± 0.11	8.08 ± 0.01	8.29 ± 0.08
20:0	0.09 ± 0.02	0.08 ± 0.02	0.00 ± 0.00	0.12 ± 0.03
21:0	0.07 ± 0.06	0.09 ± 0.04	0.06 ± 0.05	0.14 ± 0.04
22:0	0.98 ± 0.06	0.95 ± 0.06	0.83 ± 0.09	1.02 ± 0.01
23:0	0.60 ± 0.04	0.44 ± 0.02	0.45 ± 0.06	0.57 ± 0.03
24:0	0.82 ± 0.09	0.81 ± 0.07	0.84 ± 0.06	0.79 ± 0.03
∑SFA	27.73	27.88	25.72	25.05
14:1n-9	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01
15:1n-9	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.01
16:1n-7	6.11 ± 0.09	5.66 ± 0.37	5.13 ± 0.16	6.03 ± 0.04
17:1n-9	0.40 ± 0.03	0.36 ± 0.03	0.36 ± 0.04	0.45 ± 0.03
18:1n-9t	0.33 ± 0.07	0.33 ± 0.03	0.29 ± 0.05	0.40 ± 0.02
18:1n-9c	10.61 ± 0.13	10.37 ± 0.04	10.37 ± 0.04	11.03 ± 0.04
20:1n-9	0.72 ± 0.02	0.68 ± 0.04	0.69 ± 0.08	0.74 ± 0.01
22:1n-9	0.31 ± 0.01	0.28 ± 0.01	0.25 ± 0.01	0.35 ± 0.01
24:1n-9	2.00 ± 0.10	2.08 ± 0.17	1.97 ± 0.10	2.05 ± 0.16
∑MUFA	21.07	20.49	19.77	19.06
18:2n-6t[a]	0.80 ± 0.03	0.89 ± 0.04	0.82 ± 0.02	0.88 ± 0.05
18:2n-6c[a]	1.24 ± 0.05	1.13 ± 0.04	1.17 ± 0.02	1.27 ± 0.04
18:3n-6[a]	0.66 ± 0.01	0.53 ± 0.01	0.51 ± 0.01	0.69 ± 0.02
20:2n-6[a]	0.70 ± 0.03	0.66 ± 0.04	0.62 ± 0.01	0.72 ± 0.02
20:4n-6	6.40 ± 0.05	6.36 ± 0.03	6.25 ± 0.03	6.42 ± 0.02
∑n-6	9.98	9.81	9.57	9.37
18:3n-3[a]	0.31 ± 0.01	0.27 ± 0.02	0.27 ± 0.02	0.34 ± 0.02
20:3n-3[a]	1.53 ± 0.07	1.50 ± 0.01	1.50 ± 0.02	1.56 ± 0.03
20:5n-3[a]	11.12 ± 0.03	11.08 ± 0.01	11.01 ± 0.01	11.23 ± 0.07
22:6n-3[a]	7.73 ± 0.03	7.64 ± 0.01	7.52 ± 0.01	7.91 ± 0.04
∑n-3	21.04	20.69	20.49	20.30
∑PUFA	31.02	30.50	30.06	29.67
n-3/n-6	2.11	2.11	2.14	2.17

Abbreviations: ∑MUFA: monounsaturated fatty acid; ∑PUFA: polyunsaturated fatty acid; ∑SFA: saturated fatty acid.
[a]∑PUFA: 18:2n-6t, 18:3n-3, 18:3n-6, 20:2n-6t, 20:2n-6c, 20:4n-6, 20:3n-3, 20:5n-3, 22:6n-3. Results are given as mean (±SD).

Imhoff cone to keep the amount of suspended biofloc within the recommended range of 10–20 mL L^{-1} for the systems. In addition, total ammonia, nitrite-nitrogen, nitrate-nitrogen, alkalinity, and phosphate levels were determined in water samples taken at five-day intervals (Iris Visible Spectrophotometer, HI801–01 model, Hanna Instruments, USA).

2.5 | Sampling procedures and growth performance calculations

At the beginning of the experiment, the posterior digestive contents of five shrimps were sampled following the protocol for the molecular analysis of bacterial diversity. Also, the shrimp samples were taken for bacterial diversity analyses on the 45th ($n = 6$) and 90th ($n = 6$) days of the trials. For proximate analysis, at the beginning (20 shrimps) and end of the experiment ($n = 18$) shrimps were sampled. For digestive enzyme activity, non-specific immune parameters, and antioxidant enzyme activity analyses, at the beginning of the trial (20 shrimps) and at the end of the trial ($n = 24$) shrimps were sampled. At the end of the experiment, proximate analysis was also carried out from the biofloc water (50 μm plankton mesh).

Following the experiment, the growth parameters were calculated using the formulas below.

$$\text{Weight gain (WG; g/shrimp)} = \text{Final weight (g)} - \text{Initial weight (g)},$$

$$\text{Daily weight gain (DWG; g/shrimp)} = (\text{Final weight (g)} - \text{Initial weight (g)})/\text{days},$$

$$\text{Weekly weight gain (WWG; g/shrimp)} = (\text{Final weight (g)} - \text{Initial weight (g)})/\text{weeks},$$

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

$$\text{Feed conversion ratio (FCR)} = \text{Total feed given (g)}/\text{Weight gain (g)},$$

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

$$\text{Protein efficiency ratio (PER)} = \text{Weight gain (g)}/\text{Protein intake (g)},$$

$$\text{Feed conversion ratio (FCR; \%)} = \text{Feed intake (g)}/\text{Weight gain (g)},$$

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

2.6 | Proximate compositions

Experimental diet, biofloc, and shrimp were analyzed for the dry matter and crude ash levels (AOAC, 2000). The Kjeldahl method was employed to determine crude protein levels by measuring nitrogen (Nx6.25). An automated extraction method ANKOM XT-15 (Macedon, NY) was utilized to determine crude lipid levels.

The total lipid extraction of experimental diets and the whole-body shrimp were performed by chloroform/methanol method for fatty acid analysis. The lipid was methanolized using boron trifluoride (BF3) after adding methanol to the sample. 1–2 mL of the upper heptane phase was taken with a micropipette and transferred to a test tube. Fatty acid methyl esters were separated and identified by gas chromatography using a 30 m × 0.25 mm capillary column. Hydrogen was used as the carrier gas, and the temperature reached 200°C in 20 min and 220°C in

10 min, respectively. The peaks were defined according to standards (Supelco, fatty acid methyl esters, and fish oil) (AOAC, 2000).

Hydrolysis of the samples was carried out according to the hydrolysis method of an amino acid analyzer according to AOAC (2000). The sample containing 30 mg of protein was weighed and transferred to the hydrolysis tube. 10 mL of 6 N HCl was added, and the oxygen in the tube was removed by passing nitrogen gas. In the filtered sample, HCl was evaporated at 60°C under a high vacuum in the evaporator. The remaining residue was diluted with sodium citrate buffer solution with a pH of 2.2 to the desired volume and made ready for analysis.

2.7 | Digestive enzyme activity

To evaluate enzymatic activity, the hepatopancreas samples were homogenized in ice-cold water at a ratio of 1:9 (w/v) and subsequently centrifuged at 1800*g* for 30 min at 4°C. The resulting supernatant from the cold hepatopancreas was used for analysis (Shao et al., 2018). The enzymatic activities of trypsin, α-amylase, and lipase were determined using the commercial kits (Elabscience, USA).

2.8 | Non-specific immune parameters

Hemolymph was withdrawn from the basement of the second pereopods of the shrimp and pooled using sterile syringes. The shrimp was washed with sterile seawater before hemolymph puncture. After collection, the hemolymph was refrigerated at 4°C for 24 h to induce coagulation. Next, the sample was subjected to −20°C for 10 min and allowed to thaw shortly after. This process was repeated three times in succession. Subsequently, the sample was centrifuged three times at 15000*g* for 15 min. The resulting serum was collected, transferred to an Eppendorf tube, and stored at −20°C until analysis (Sabry Neto & Nunes, 2015).

Phenoloxidase (PO) was determined by the spectrophotometric method based on the measurement of L-dihydroxyphenylalanine (L-DOPA) formation (Hernández-López, 1996). The total hemocyte count (THC) was estimated by an optical microscope using a Neubauer hemocytometer chamber. The production of superoxide anions was measured by determining the respiratory burst activity (RB) of the hemocytes, which involved reducing nitro blue tetrazolium (NBT) to formazan (Song & Hsieh, 1994). For the measurements of activities of the total anti-oxidative capacity (T-AOC), superoxide dismutase (SOD), glutathione, alkaline phosphatase (ALP), acid phosphatase (ACP) in serum, the kits (Sigma-Aldrich) were used according to the manufacturer's protocols.

2.9 | Analysis of microbial diversity in shrimp gut

The posterior digestive contents of shrimps were suspended in 2 mL Fix RNA (EuRx) solution in a sterile tube. DNA extraction in posterior digestive contents was performed directly from feces using the EuRx Tissue & Bacterial DNA Purification Kit (EuRx) according to the manufacturer's protocol. The quality and quantity of extracted DNA were measured with the SPECTROstar Nano Spectrometer device. 16S Universal bacterial primers encoded 16S Forward and 16S Reverse were utilized to enrich each sample's 16S rDNA V3 and V4 regions. Amplification of 16S rDNA genes was performed with the following primers: 16sV3F (ACTCCTACGGGAGGCAGCAGT) and 16sV3R (ACCGCGGCTGCTGGCAC).

The primer set offering the highest efficacy was selected for use in the experiment (Çelik & Keskin, 2022). PCR was performed with a primary heating step for 2 min at 94°C, followed by 35 cycles of denaturation for 1 min at 94°C, annealing for 45 s at 53°C, and extension for 25 s at 72°C, then followed by a final extension step for 5 min at 72°C. Each 10 μL reaction mixture contained 2 μL of 5× Promega Colorless GoTaq Flexi buffer, 0.5 μL DNA,

1.5 μL of 25 mM $MgCl_2$, 0.8 μL of 3.2 μM dNTP, 0.5 μL of each primer (5 pmol/mL), 0.05 μL of Taq DNA polymerase, and sterile water to a final volume of 10 μL.

Sequencing libraries were generated using Nextera DNA Prep Library Prep Kit (Illumina, San Diego, CA, USA) following the manufacturer's recommendations, and index codes were added. The samples were then sequenced bidirectionally (2 × 150bp) on an Illumina iSeq 100 platform. ".fastq" files that obtained from the iSeq 100 device were analyzed using the "ObiTools" package (Boyer et al., 2016) After reads including taxonomic profiling, alignment, and identification were completed the visualization, analysis, and identification of OTU groups in each sample and reporting of the resulting data were applied using Geneious Prime and SILVAngs (Kaya et al., 2020; Quast et al., 2012).

2.10 | Data analysis

Analyzes were performed on the data sets using the SPSS 17 (Chicago, IL, USA) statistical package program. Before analysis (analysis of variance, Duncan), assumptions were tested. First, the skewness and kurtosis statistics were taken into account for all groups and were examined in terms of normality assumptions, and the homogeneity of the variances was evaluated with the Levene test. All data were given as mean ± standard deviation. Statistical significance was regarded as $p < 0.05$. The broken-line model was used to determine protein requirements. Estimating the optimum dietary protein requirement was predicted from a segmented regression model of the SGR against the dietary protein level using broken-line model analysis (Oosterbaan, 1994).

3 | RESULTS

Water quality parameters of the biofloc rearing mediums are summarized in Table 4. All water quality parameters remained desired ranges for BFT and there were no significant differences among the groups.

Increasing dietary protein levels significantly ($p < 0.05$) enhanced FW, WG, and SGR indices, as presented in Table 5. FW was significantly ($p < 0.05$) higher in the 38P and 35P than in other treatments. 38P had an intermediate effect on WG and SGR parameters which indicated they were similar to 35P and 32P treatments ($p > 0.05$). PER of shrimp was improved in decreasing protein levels, with the highest in the 29P group ($p < 0.05$). However, FCR and SR were not affected by different dietary protein levels ($p > 0.05$).

TABLE 4 Water quality parameters of rearing waters at 90 days culture period.

Parameters	38P	35P	32P	29P	*N*
Water temperature (°C)	28.24 ± 0.26	28.29 ± 0.29	28.24 ± 0.31	28.24 ± 0.20	90
Dissolved oxygen (mg L^{-1})	6.56 ± 0.23	6.42 ± 0.28	6.46 ± 0.32	6.45 ± 0.35	90
pH	8.07 ± 0.17	7.99 ± 0.11	8.02 ± 0.13	7.96 ± 0.19	90
Nitrite (mg L^{-1})	0.36 ± 0.02	0.35 ± 0.01	0.35 ± 0.01	0.35 ± 0.01	18
Nitrate (mg L^{-1})	10.98 ± 0.88	10.92 ± 0.75	10.66 ± 0.63	10.75 ± 0.64	18
Ammonia (mg L^{-1})	0.42 ± 0.03	0.42 ± 0.03	0.42 ± 0.03	0.41 ± 0.02	18
Alkalinity (mg L^{-1})	104.06 ± 4.93	105.11 ± 5.55	104.33 ± 5.74	104.78 ± 5.33	18
Phosphate (mg L^{-1})	0.70 ± 0.04	0.70 ± 0.03	0.71 ± 0.03	0.71 ± 0.03	18
Biofloc volume (ml L^{-1})	13.50 ± 1.20	13.35 ± 1.33	13.52 ± 1.41	13.10 ± 1.45	90

Note: *N*: sampling number.

TABLE 5 Growth performance of *Farfantepenaeus aztecus* fed diets with decreasing protein levels.

Parameters	38P	35P	32P	29P
IW (g)	0.841 ± 0.032	0.840 ± 0.038	0.839 ± 0.054	0.839 ± 0.044
FW (g)	10.016 ± 0.489^{b}	10.010 ± 0.665^{b}	9.702 ± 0.269^{a}	9.357 ± 0.320^{a}
WG (g)	9.088 ± 0.219^{bc}	9.270 ± 0.075^{c}	8.863 ± 0.077^{b}	8.518 ± 0.053^{a}
DWG (g)	0.101 ± 0.002^{bc}	0.103 ± 0.001^{c}	0.098 ± 0.001^{b}	0.095 ± 0.001^{a}
WWG (g)	0.707 ± 0.017^{bc}	0.721 ± 0.006^{c}	0.689 ± 0.006^{b}	0.662 ± 0.004^{a}
WG (%)	1081.216 ± 26.897^{bc}	1103.205 ± 6.393^{c}	1056.989 ± 8.908^{b}	1015.060 ± 7.229^{a}
SGR (% day^{-1})	2.743 ± 0.025^{bc}	2.764 ± 0.006^{c}	2.720 ± 0.009^{b}	2.679 ± 0.007^{a}
PER	1.930 ± 0.068^{a}	2.103 ± 0.075^{b}	2.282 ± 0.057^{c}	2.411 ± 0.045^{d}
FCR	1.365 ± 0.048^{a}	1.360 ± 0.049^{a}	1.370 ± 0.034^{a}	1.431 ± 0.027^{a}
SR (%)	88.333 ± 7.638^{a}	86.667 ± 7.638^{a}	85.000 ± 5.000^{a}	85.000 ± 5.000^{a}

Note: Results are given as mean (±SD). Values in the same row with different superscripts indicate significant differences ($p < 0.05$).
Abbreviations: DWG, Daily weight gain; FCR, feed conversion ratio; FW, final weight; IW, initial weight; PER, protein efficiency ratio; SGR, specific growth rate; SR, survival rate; WG, weight gain; WWG, weekly weight gain.

Broken-line analysis of the SGR showed that 34.40% dietary protein had the optimum effect on shrimp's specific growth rate under the BFT culture conditions. The yield reduced after this threshold (breakpoint, knot) (Figure 1).

On the other hand, no breakpoint was observed in the segmented regression analysis for the optimum protein levels in the feed for the highest protein efficiency ratio. In other words, as shown in Figure 2, the model suggests BPx = Xmin. In this equation, BPx stands for the breakpoint on the *x*-axis and Xmin represents the minimum value of the independent variable. The results suggest that the breakpoint (BPx) equals the minimum value of the dietary protein level (%). This implies that the segmented regression model has no breakpoint and the highest protein efficiency rate is located at the minimum value of the dietary protein level with 29%.

The proximate composition of the crude protein was different in the 38P and 35P treatments, indicating that whole-body crude protein was affected by different dietary protein levels in BFT ($p < 0.05$). However, crude lipids showed no significant difference among the groups ($p > 0.05$) (Table 6).

Proximate analysis of biofloc is shown in Table 7. The filtered bioflocs of the rearing waters showed no differences among the groups.

Whole-body fatty acid analysis of the shrimp fed with different dietary protein levels is presented in Table 8. An apparent increase in SFA and MUFA compositions was noticed in shrimp fed at 38% and 35% crude protein levels ($p < 0.05$). Total n-6 fatty acids in the whole body increased trend when the diet contained a 35% crude protein level ($p < 0.05$). PUFA and total n-3 fatty acids had the highest amounts in the shrimp fed with 35% crude protein in the diet ($p < 0.05$).

As shown in Table 9, THC and ACP indices were significantly ($p < 0.05$) higher in the 38P and 35P than in the 32P and 29P treatments. 38P showed the highest RB and Glutathione activities of all treatments ($p < 0.05$). The highest ALP activities were observed in the 32P group, and there was a statistical difference compared to the 38P group ($p < 0.05$). However, PO and SOD activities did not indicate any statistical difference among the treatments ($p > 0.05$). While the highest T-AOC activities were recorded in 38P, 35P had an intermediate effect on T-AOC activities which stated there were similar compared to other treatments ($p > 0.05$).

Dietary protein levels significantly ($p < 0.05$) affected the protease activity of shrimp, with the highest value in the 38P treatment. 32P had an intermediate effect on protease activities with the 35P and 29P groups

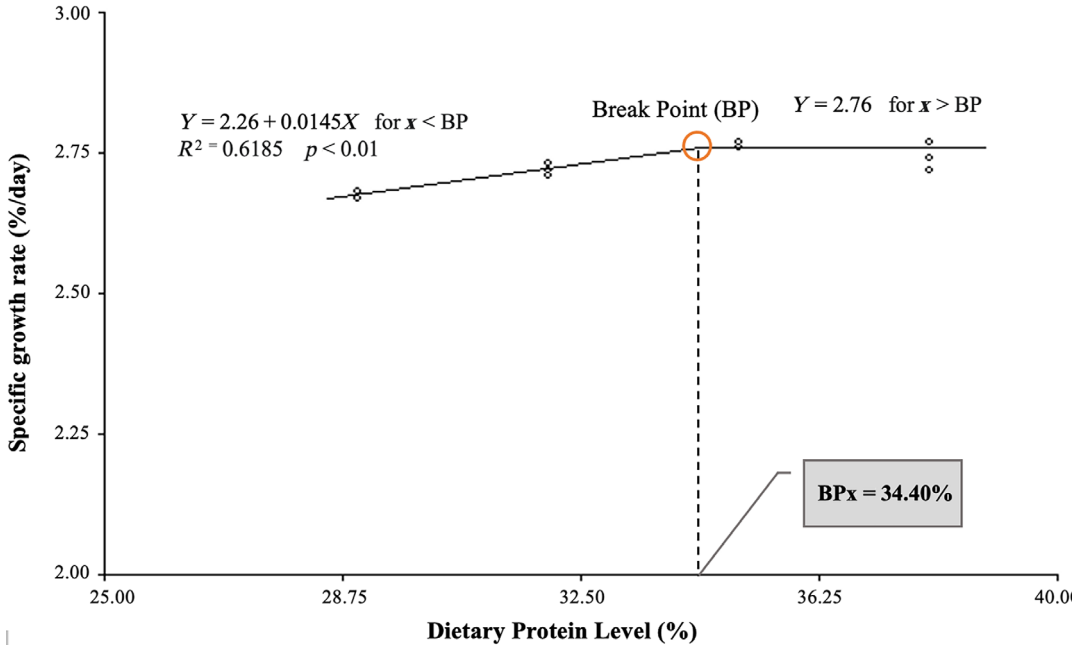

FIGURE 1 Relationship between dietary protein level and specific growth rate of shrimp fed the experimental diets.

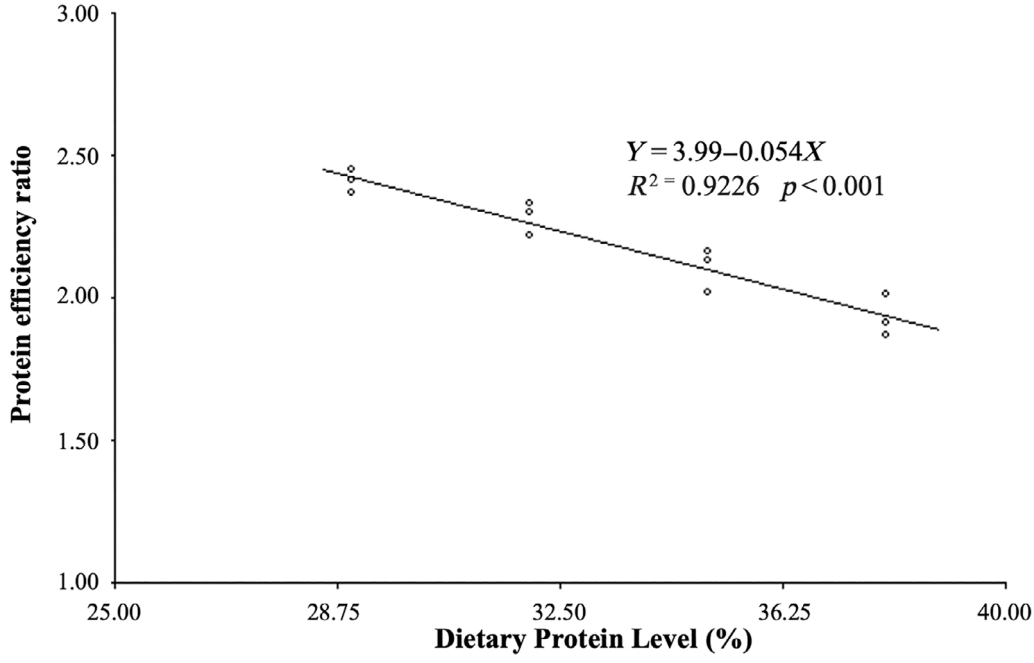

FIGURE 2 Relationship between dietary protein level and protein efficiency ratio of shrimp fed the experimental diets.

($p > 0.05$). 38P and 35P groups were higher than the 32P and 29P groups in terms of amylase activities ($p < 0.05$). The lipase activity was significantly ($p < 0.05$) increased in the 38P, 35P, and 32P, whereas the 29P was lower (Table 10).

TABLE 6 Whole body composition of *Farfantepenaeus aztecus* fed diets with decreasing protein levels (% dry weight).

	Initial	38P	35P	32P	29P
Dry matter	21.01 ± 0.61	24.22 ± 0.99[a]	25.22 ± 0.63[a]	24.82 ± 1.19[a]	23.45 ± 0.55[a]
Crude ash	10.59 ± 0.18	14.92 ± 0.17[a]	14.93 ± 0.14[a]	15.41 ± 0.44[ab]	15.84 ± 0.43[b]
Crude lipid	2.78 ± 0.17	3.58 ± 0.08[a]	3.93 ± 0.22[a]	3.58 ± 0.23[a]	3.76 ± 0.06[a]
Crude protein	70.87 ± 0.36	76.24 ± 0.37[b]	76.52 ± 0.51[b]	74.95 ± 0.51[a]	74.72 ± 0.44[a]

Note: Results are given as mean (±SD). Values in the same row with different superscripts indicate significant differences except for the initial column ($p < 0.05$).

TABLE 7 Proximate composition of biofloc (%).

	38P	35P	32P	29P
Crude ash	48.33 ± 0.21	48.23 ± 0.32	48.37 ± 0.40	48.37 ± 0.40
Crude lipid	0.38 ± 0.02	0.38 ± 0.02	0.35 ± 0.04	0.37 ± 0.02
Crude protein	23.53 ± 0.31	23.47 ± 0.32	23.47 ± 0.35	23.47 ± 0.40

The bacterial composition in the shrimp gut microbiota is presented in Figure 3. The most abundant phyla in the initial gut microbiota of shrimp were determined as Proteobacteria (54%), Firmicutes (22%), Bacteroidota (10%), Actinobacteria (7%), Verrucomicrobiota (2%), respectively. In the mid of the experiment (45 days), Proteobacteria showed the most intensity among the groups, and Bacteroidota was the second most abundant phylum in the 38P, 35P, and 29P groups, while Firmicutes was the second most abundant phylum in the 32P group. At the end of the experiment, Proteobacteria remained the most abundant bacteria in the 38P, 35P, 32P, and 29P groups, 83, 68, 73, and 76%, respectively. Firmicutes was the second most common bacteria in the 38P (5%) and 35P (17%) groups, while Bacteroidota was the second most common in the 32P (12%) and 29P (13%) groups.

The species-level bacterial density (>1%) of the shrimp gut microbiota at the initial, mid, and end of the experiment is presented in Figure 4. Initial shrimp gut microbiota composed most intensity with *Cribrihabitans pelagius* (16.69%), *Spongiimonas flava* (10.22%), *Ruegeria marisrubri* (6.12%), *Vibrio hyugaensis* (5.73%), *Ruegeria meonggei* (3.65%), *Pararhizobium haloflavum* (3.60%), *Vibrio alginolyticus* (2.19%), *Vibrio hangzhouensis* (2.02%), *Amylibacter lutimaris* (1.80%), *Tritonibacter multivorans* (1.80%), *Demequina litorisediminis* (1.80%), *Vibrio tapetis* (1.57%), *Microvirga massiliensis* (1.35%), *Vibrio barjaei* (1.01%), and *Litorisediminivivens gilvus* (1.01%). In the 38P group, *Cribrihabitans pelagius*, *Spongiimonas flava*, and *Ruegeria marisrubri* densities decreased at the end of the trial, while *Vibrio hyugaensis* (19.91%), *Vibrio barjaei* (12.44%), and *Vibrio jasicida* (11.51%) were the most common bacteria. Similarly, in the 35P and 32P groups, *Vibrio hyugaensis* (20.49% and 17.63%), *Vibrio barjaei* (14.71% and 10.64%), and *Vibrio jasicida* (13.62% and 9.54%) were the most common bacteria at the end of the trial. In the 29P group, the most common bacteria were determined as *Vibrio hyugaensis* (10.81%), *Vibrio tapetis* (9.04%), and *Vibrio jasicida* (6.67%).

4 | DISCUSSION

Previous studies have reported that reduction of dietary protein content under BFT conditions can be achieved in shrimp without negatively affecting growth. It has been reported that BFT conditions can reduce the protein content of the diet by at least 5% in the culture of *L. vannamei* (Olier et al., 2020). In our study, shrimp fed 3% less crude protein (5.8% less fish meal) with BFT conditions showed similar growth performance to shrimp cultured on a 38% protein diet. Furthermore, broken line regression analysis of SGR data indicated that the optimal dietary protein level

TABLE 8 Whole body fatty acid composition of *Farfantepenaeus aztecus* fed diets with decreasing protein levels (mg g^{-1}).

Fatty acid	Initial	38P	35P	32P	29P
12:0	0.01 ± 0.01	0.09 ± 0.02^{c}	0.07 ± 0.01^{b}	0.06 ± 0.01^{ab}	0.04 ± 0.01^{a}
13:0	0.10 ± 0.01	0.15 ± 0.01^{b}	0.19 ± 0.03^{c}	0.13 ± 0.01^{b}	0.08 ± 0.01^{a}
14:0	1.49 ± 0.04	1.53 ± 0.03^{b}	1.67 ± 0.01^{c}	1.44 ± 0.02^{a}	1.56 ± 0.03^{b}
15:0	0.69 ± 0.02	0.61 ± 0.02^{b}	0.76 ± 0.02^{c}	0.54 ± 0.02^{a}	0.65 ± 0.02^{b}
16:0	12.33 ± 0.61	13.12 ± 0.13^{b}	13.03 ± 0.48^{b}	11.65 ± 0.44^{a}	11.25 ± 1.00^{a}
17:0	0.95 ± 0.17	1.01 ± 0.16^{a}	1.11 ± 0.16^{a}	1.19 ± 0.12^{a}	1.02 ± 0.18^{a}
18:0	6.16 ± 0.06	8.26 ± 0.08^{b}	8.31 ± 0.10^{b}	8.05 ± 0.11^{a}	8.03 ± 0.03^{a}
20:0	0.00	0.08 ± 0.03^{b}	0.04 ± 0.05^{ab}	0.03 ± 0.03^{ab}	0.00^{a}
21:0	0.00	0.06 ± 0.04^{a}	0.02 ± 0.03^{a}	0.04 ± 0.06^{a}	0.02 ± 0.02^{a}
22:0	0.73 ± 0.06	0.81 ± 0.02^{a}	0.79 ± 0.15^{a}	0.84 ± 0.05^{a}	0.72 ± 0.14^{a}
23:0	0.38 ± 0.02	0.44 ± 0.04^{a}	0.44 ± 0.02^{a}	0.37 ± 0.03^{a}	0.41 ± 0.07^{a}
24:0	0.79 ± 0.04	0.78 ± 0.01^{a}	0.75 ± 0.06^{a}	0.82 ± 0.07^{a}	0.80 ± 0.08^{a}
∑SFA	23.63 ± 0.46	26.94 ± 0.31^{b}	27.16 ± 0.61^{b}	25.16 ± 0.49^{a}	24.57 ± 0.75^{a}
14:1n-9	0.00	0.01 ± 0.01^{a}	0.00^{a}	0.00^{a}	0.00^{a}
15:1n-9	0.00	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}	0.00^{a}	0.00^{a}
16:1n-7	5.35 ± 0.13	5.94 ± 0.05^{c}	6.05 ± 0.05^{c}	5.38 ± 0.19^{b}	5.05 ± 0.20^{a}
17:1n-9	0.29 ± 0.07	0.38 ± 0.04^{b}	0.38 ± 0.01^{b}	0.30 ± 0.05^{a}	0.35 ± 0.02^{a}
18:1n-9t	0.33 ± 0.02	0.32 ± 0.01^{a}	0.32 ± 0.06^{a}	0.30 ± 0.04^{a}	0.31 ± 0.06^{a}
18:1n-9c	10.26 ± 0.09	10.55 ± 0.02^{b}	10.49 ± 0.06^{b}	10.30 ± 0.02^{a}	10.33 ± 0.05^{a}
20:1n-9	0.57 ± 0.02	0.66 ± 0.04^{a}	0.71 ± 0.03^{a}	0.66 ± 0.05^{a}	0.67 ± 0.09^{a}
22:1n-9	0.23 ± 0.03	0.25 ± 0.03^{a}	0.25 ± 0.02^{a}	0.22 ± 0.04^{a}	0.25 ± 0.05^{a}
24:1n-9	1.82 ± 0.03	1.90 ± 0.10^{a}	1.88 ± 0.18^{a}	1.88 ± 0.10^{a}	1.88 ± 0.16^{a}
∑MUFA	18.84 ± 0.13	20.03 ± 0.05^{b}	20.10 ± 0.13^{b}	19.05 ± 0.14^{a}	18.84 ± 0.39^{a}
18:2n-6t*	0.56 ± 0.03	0.65 ± 0.02^{a}	0.69 ± 0.03^{a}	0.68 ± 0.06^{a}	0.71 ± 0.06^{a}
18:2n-6c*	0.93 ± 0.06	1.08 ± 0.01^{a}	1.17 ± 0.05^{c}	1.09 ± 0.02^{ab}	1.14 ± 0.02^{bc}
18:3n-6*	0.36 ± 0.01	0.45 ± 0.03^{a}	0.49 ± 0.03^{a}	0.46 ± 0.02^{a}	0.44 ± 0.03^{a}
20:2n-6*	0.42 ± 0.03	0.63 ± 0.05^{ab}	0.66 ± 0.04^{b}	0.59 ± 0.07^{ab}	0.55 ± 0.03^{a}
20:4n-6	6.23 ± 0.13	6.33 ± 0.06^{b}	6.37 ± 0.06^{b}	6.25 ± 0.08^{ab}	6.20 ± 0.03^{a}
∑n-6	8.50 ± 0.14	9.14 ± 0.03^{a}	9.38 ± 0.09^{b}	9.07 ± 0.16^{a}	9.04 ± 0.15^{a}
18:3n-3*	0.19 ± 0.02	0.25 ± 0.02^{a}	0.26 ± 0.04^{a}	0.22 ± 0.01^{a}	0.24 ± 0.04^{a}
20:3n-3*	1.39 ± 0.03	1.46 ± 0.08^{a}	1.50 ± 0.07^{a}	1.47 ± 0.02^{a}	1.44 ± 0.04^{a}
20:5n-3*	10.69 ± 0.02	11.00 ± 0.09^{b}	11.04 ± 0.07^{b}	10.94 ± 0.07^{b}	10.55 ± 0.07^{a}
22:6n-3*	7.65 ± 0.04	7.58 ± 0.01^{b}	7.51 ± 0.06^{ab}	7.52 ± 0.06^{ab}	7.44 ± 0.04^{a}
∑n-3	19.92 ± 0.06	20.30 ± 0.11^{bc}	20.31 ± 0.04^{c}	20.15 ± 0.10^{b}	19.67 ± 0.06^{a}
∑PUFA	28.42 ± 0.21	29.44 ± 0.14^{b}	29.69 ± 0.07^{c}	29.22 ± 0.09^{b}	28.71 ± 0.18^{a}
n-3/n-6	2.34 ± 0.42	2.22 ± 0.01^{a}	2.17 ± 0.02^{a}	2.22 ± 0.05^{a}	2.17 ± 0.03^{a}

Note: Results are given as mean (±SD). Values in the same row with different superscripts indicate significant differences except for the initial column ($p < 0.05$).

Abbreviations: ∑MUFA: monounsaturated fatty acid; ∑PUFA: polyunsaturated fatty acid; ∑SFA: saturated fatty acid.

*∑PUFA: 18:2n-6t, 18:3n-3, 18:3n-6, 20:2n-6t, 20:2n-6c, 20:4n-6, 20:3n-3, 20:5n-3, 22:6n-3.

TABLE 9 Non-specific immune parameters and antioxidant enzyme activities of *Farfantepenaeus aztecus* fed diets with decreasing protein levels.

	Initial	38P	35P	32P	29P
THC ($\times 10^6$ cells mL^{-1})	2.07 ± 0.38	2.60 ± 0.10^b	2.57 ± 0.08^b	1.79 ± 0.01^a	1.77 ± 0.15^a
PO (U mL^{-1})	0.13 ± 0.02	0.15 ± 0.01^a	0.15 ± 0.01^a	0.15 ± 0.01^a	0.13 ± 0.02^a
RB (10 μL^{-1})	0.29 ± 0.01	0.73 ± 0.12^b	0.43 ± 0.03^a	0.37 ± 0.12^a	0.39 ± 0.11^a
ALP (U 100 mL^{-1})	3.80 ± 0.20	4.28 ± 0.25^a	4.50 ± 0.30^{ab}	4.70 ± 0.00^b	4.57 ± 0.06^{ab}
ACP (U 100 mL^{-1})	10.83 ± 0.06	12.37 ± 0.06^b	12.40 ± 0.17^b	11.02 ± 0.03^a	11.17 ± 0.21^a
SOD (U mL^{-1})	30.67 ± 1.15	32.33 ± 0.58^a	30.67 ± 1.15^a	30.67 ± 1.15^a	30.67 ± 1.15^a
T-AOC (IU mL^{-1})	8.33 ± 0.58	10.33 ± 0.58^b	9.67 ± 0.58^{ab}	8.67 ± 1.15^a	8.67 ± 0.58^a
Glutathione (IU mL^{-1})	1.20 ± 0.10	1.43 ± 0.06^c	1.27 ± 0.12^b	1.17 ± 0.06^{ab}	1.03 ± 0.06^a

Note: Results are given as mean (±SD). Values in the same row with different superscripts indicate significant differences except for the initial column ($p < 0.05$).
Abbreviations: ACP, acid phosphatase; ALP, alkaline phosphatase; PO, phenoloxidase activity; RB, respiratory burst activity; SOD, superoxide dismutase; T-AOC, total antioxidant capacity; THC, total hemocyte count.

TABLE 10 Digestive enzyme activity of *Farfantepenaeus aztecus* fed diets with decreasing protein levels (U/g).

Parameters	Initial	38P	35P	32P	29P
Protease	409.67 ± 29.14	477.33 ± 6.43^c	466.67 ± 5.78^b	460.33 ± 5.51^{ab}	454.67 ± 4.51^a
Amylase	212.33 ± 9.29	275.67 ± 0.58^b	276.67 ± 2.89^b	230.67 ± 1.15^a	229.33 ± 1.15^a
Lipase	249.33 ± 2.08	289.33 ± 1.15^b	286.67 ± 5.77^b	283.33 ± 5.77^b	273.33 ± 2.89^a

Note: Results are given as mean (±SD). Values in the same row with different superscripts indicate significant differences except for the initial column ($p < 0.05$).

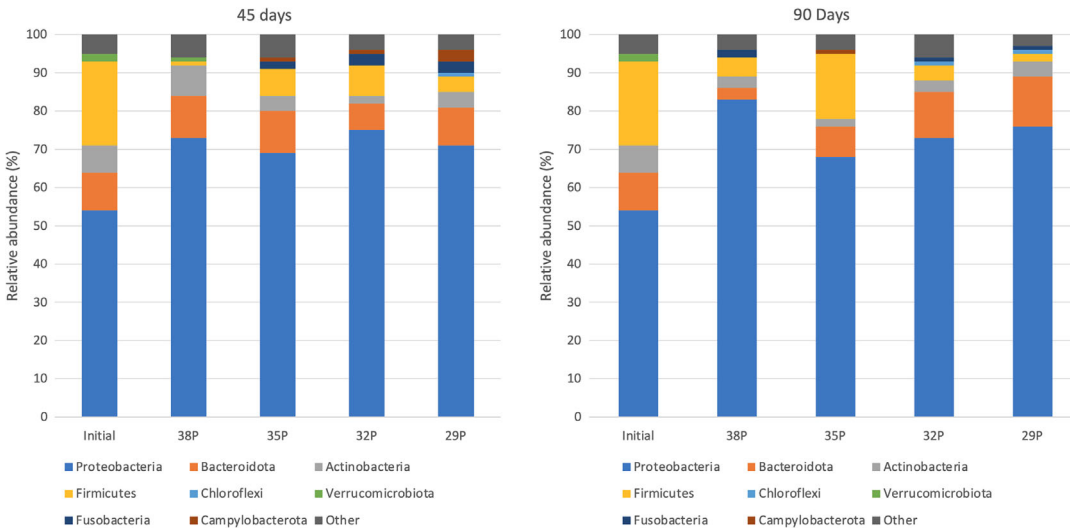

FIGURE 3 Relative abundance of most frequently identified bacterial phylum (>1% of total sequences) in shrimp guts at different dietary protein levels in biofloc technology.

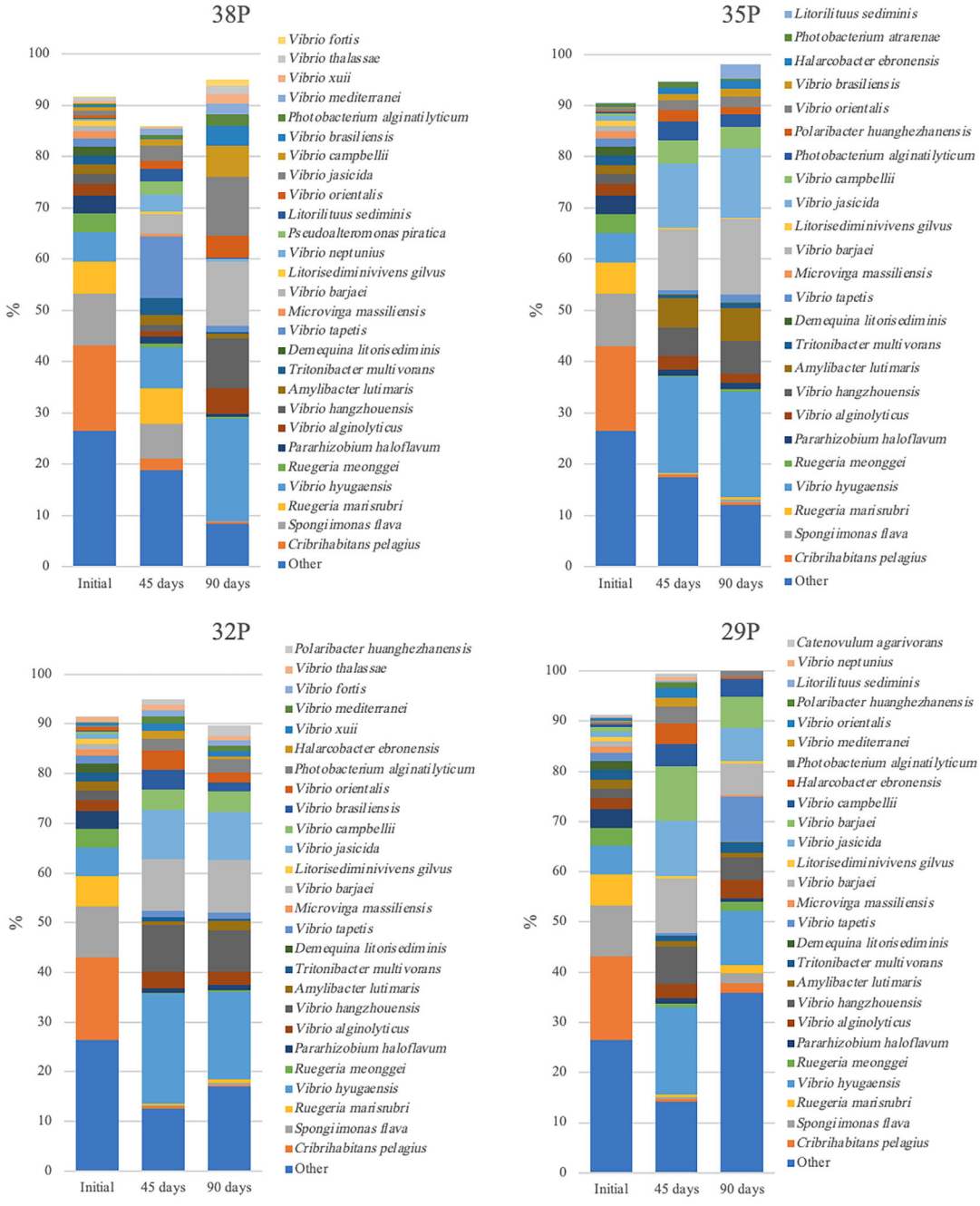

FIGURE 4 Relative abundance of most frequently identified bacterial species (>1% of total sequences) in shrimp guts at different dietary protein levels in biofloc technology.

for the shrimp was 34.40%, in agreement with the growth performance data. However, 6% and 9% less protein content in the diet negatively affected shrimp growth performance in BFT culture conditions. Similarly, in a study conducted by Pinho and Emerenciano (2021), the production performances of *L. vannamei* were significantly reduced as a result of a 10% reduction in dietary protein, with the group treated with the highest protein level (35% of CP)

having higher values than shrimp fed with 25% CP. It was also reported that the growth performance of *P. monodon* fed with 32% protein in the diet under BFT conditions (with molasses as a carbon source) decreased compared to shrimp fed with 40% protein (Kumar et al., 2017). In the current study, biofloc as an extra food was probably insufficient to make up for the dietary protein difference below 35%. The optimal dietary protein level in BFT conditions may vary depending on the species, carbon source used, biofloc composition, and water quality. In our study, all water quality parameters remained in the desired ranges for BFT and shrimp culture conditions (Avnimelech, 2015).

This study provides the first information on the effect of different dietary protein levels on shrimp's whole-body fatty acids composition in BFT. Whole body fatty acid composition depends on the fatty acid profile of the diet (Tazikeh et al., 2020). Our data demonstrated no considerable differences in the whole-body fatty acid contents including MUFA, PUFA and SFA of *F. aztecus* fed with different dietary protein levels in the diet.

Like other crustaceans, shrimps rely on non-specific immunity because of not have an adaptive immune system (Bachère, 2000). Circulating hemocytes and PO activity have a critical role in the defense mechanism of shrimp (Rodriguez & Le Moullac, 2000), while antioxidant enzymes protect cells from oxidative stress in host-pathogen interplay (Holmblad & Söderhäll, 1999). The present study showed that increasing protein contents in the diet had a better immune response concerning THC and ACP indices, and the diet with the highest protein content improved RB and Glutathione activities. This indicates that achieving enhanced immunity in the shrimp by consuming the microbial floc in situ could require a high-protein diet for these parameters. But reducing the dietary protein level from 38% to 29% did not negatively affect PO, ALP and SOD activities in the present study. Reducing the protein in the diet from 40% to 24% in *L. vannamei* had a negative effect on the immune response (Panigrahi et al., 2019). On the contrary, in a study conducted by Xu and Pan (2014) the THC and immune responses of *L. vannamei* were not affected by decreasing the dietary protein levels from 35% to 20% under BFT, while decreasing trends in the T-AOC in both the plasma and the hepatopancreas were found as dietary protein levels decreased. The bioactive components in biofloc may affect the health status of shrimps, especially on immune and antioxidant indices with the differential dietary protein levels. Therefore, optimization of dietary protein content is essential for maintaining immunity.

Digestive enzymes are critical for nutrient digestion, and the digestive capacity, nutritional status, and growth performance of the cultured species are directly affected by their activities. Protein and lipid contents of the diet can affect the endogenous enzymes of the species (Méndez-Martínez et al., 2018). As a food supplement, biofloc can improve the utilization of the feed and growth by stimulating digestive enzyme activity (G Liu et al., 2017). The present study generally revealed that dietary protein levels influenced protease, amylase, and lipase activities in the diet under BFT conditions. However, except for protease, amylase, and lipase activities of the shrimp were not negatively affected in the diet with 3% lower protein content compared to 38% protein. This may explain why similar growth was achieved in the 38P and 35P groups. Previously, it has been shown that the level of protein in the diet has effects on the digestive enzyme activity of shrimp (Talukdar et al., 2021; Xia et al., 2010) but more work is needed to fully clarify the effects of the dietary protein levels on the digestive enzyme activities with BFT.

As with the fish gut microbiome, the shrimp gut microbiome similarly contains about 90% Bacteroidetes, Firmicutes, and Proteobacteria (Ghanbari et al., 2015). However, the gut microbiome of shrimp varies depending on certain factors which may be as a result of variations in species, developmental stage, water temperature, and diet (Rajeev et al., 2021). Our study demonstrated that the most common phylum of the shrimp gut microbiota in all groups was distributed in Proteobacteria, followed by Firmicutes for 38P and 35P groups and by Bacteroidetes for 32P and 29P groups. Qiao et al. (2017) pointed out that the abundance of Firmicutes in the shrimp gut microbiota was sensitive to carbohydrates in the diet. This may explain how increasing carbohydrate contents in the diet affected Firmicutes abundance in the current study.

Although there are pathogenic strains, most Vibrio spp. are known to be non-pathogenic for the host (Yu et al., 2018). The virulence of Vibrio species may vary depending on culture conditions or induced quorum-sensing regulation (Zhang et al., 2017). Our study data showed a significant increase of Vibrio in the shrimp gut under BFT conditions. Similar to our study, Vibrio enrichment was also reported in the *L. vannamei* gut microbiota under biofloc

conditions (Tepaamorndech et al., 2020). Gustilatov et al. (2022) stated that BFT induced a decrease in the pathogenicity of *V. parahaemolyticus*, regardless of density. Thus, the positive roles of biofloc with gut microbiome may be reflected in the healthy growth of shrimp throughout the culture period.

5 | CONCLUSION

The results of the present study showed that with a biofloc system, reducing the protein levels in the diet to a certain extent may be effective. However, biofloc may not be a substitute for suboptimal protein levels in the diet. In the present study, we suggested that for brown shrimp culture in a biofloc system, dietary protein levels could be reduced from 380 to 350 g kg^{-1} without negatively affecting healthy growth. With optimum protein levels in the diet, approaches that can improve the efficiency of the biofloc and healthy rearing of brown shrimp is also a matter of further research.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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