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Effects of yeast extract and sweet red pepper powder applied to culture water on the rearing performance of *Penaeus vannamei* in a biofloc system

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Abstract

The effects of functional materials applied directly to biofloc culture water remain poorly understood. This study evaluated the individual and combined effects of yeast extract (YE) and dried sweet red pepper powder (SRP) on Pacific white shrimp (*Penaeus vannamei*) reared in a biofloc system. Shrimp with an initial mean weight of approximately 1.50 g were stocked at 20 individuals per 45-L tank and cultured for 60 days. YE and SRP were applied daily at 0, 1, or 3 mg L^{-1} in a 3×3 factorial design with three replicate tanks per treatment. Growth, feed conversion ratio, survival, total haemocyte count, whole-body composition, water quality, biofloc characteristics, and total culturable heterotrophic bacterial density were evaluated. YE significantly affected final weight, weight gain, specific growth rate, and feed conversion ratio. Marginal means showed that 3 mg L^{-1} YE increased final weight, weight gain, and specific growth rate compared with YE-free treatments, while both 1 and 3 mg L^{-1} YE improved feed conversion ratio. Final weight increased from 7.77 to 8.45 g, while weight gain increased from 6.27 to 6.94 g $shrimp^{-1}$. SRP had no significant main effect on growth, feed utilisation, or survival, and no significant YE × SRP interaction was detected. Neither additive significantly affected total haemocyte count, culturable heterotrophic bacterial density, shrimp whole-body composition, or biofloc proximate composition. Water-quality variables and biofloc volume remained within narrow ranges among treatments. These findings indicate that waterborne YE may improve growth and feed utilisation in *P. vannamei* under BFT conditions without detectable changes in basic biofloc composition or culturable bacterial density. In contrast, SRP provided no additional production benefit at the tested levels.

Keywords: Biofloc technology, Functional additives, Yeast extract, Sweet red pepper, Growth performance, Pacific white shrimp

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Introduction

Biofloc technology (BFT) is an aquaculture approach in which nitrogenous compounds and other dissolved nutrients entering the culture environment are converted into microbial biomass through the controlled addition of organic carbon. The bioflocs produced during this process contribute to water quality management and may also provide an additional food source for cultured animals. The successful operation of BFT systems therefore depends on the combined management of carbon input, the carbon-to-nitrogen ratio, feeding practices, stocking density, and microbial development (Crab *et al*., 2012; Emerenciano *et al*., 2017; El-Sayed, 2021; McCusker *et al*., 2023). The nutritional and microbial properties of bioflocs are not constant but may vary according to the carbon source, nutrient load, carbon-to-nitrogen ratio, and environmental conditions. Consequently, different organic or functional materials introduced into a BFT system may influence both the culture environment and the performance of the cultured animals.

In shrimp aquaculture, BFT can provide important advantages by retaining nutrients within microbial biomass, controlling inorganic nitrogen compounds, and creating a biologically active culture environment. However, the effects of BFT are also influenced by feeding rate, feed composition, stocking density, and microbial management practices. Previous studies have shown that changes in feed input, carbon supplementation, and feed additives may affect the bacterial composition of bioflocs, water quality, growth performance, and the physiological condition of cultured animals (Pérez-Fuentes *et al*., 2018; Rodrigues *et al*., 2018; Hassaan *et al*., 2018; Said *et al*., 2024; Genc *et al*., 2024a,b). Biofloc-associated microbial communities may also interact with the intestinal microbiota of shrimp and influence their nutritional and health status (Cardona *et al*., 2016; Hostins *et al*., 2017; Valente *et al*., 2020; Panigrahi *et al*., 2022). These findings indicate that organic or functional materials added to BFT systems should be evaluated not only in terms of shrimp growth but also in relation to biofloc composition, microbial abundance, and water quality.

Microalgae, natural food organisms, plant-derived materials, and different carbon sources have been used to modify the nutritional or microbial properties of bioflocs. These applications have been reported to influence biofloc development, water quality, microbial community structure, and the performance of cultured animals (Abbaszadeh *et al*., 2022; Silva *et al*., 2022). Similarly, probiotics, synbiotics, and other functional feed additives have been investigated for their effects on water quality, microbial communities, growth performance, and immune responses in shrimp and other aquaculture species (Rodrigues *et al*., 2018; Hussain *et al*., 2021; Qiu *et al*., 2023; Genc *et al*., 2024a,b; Santos *et al*., 2024). However, the effects of these materials depend not only on their

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chemical composition but also on their application route and dose. Therefore, a material incorporated into the diet cannot be assumed to have the same effects on biofloc structure and microbial processes when applied directly to the culture water.

Yeast-derived products are considered promising functional materials in aquaculture because they may contain proteins, peptides, nucleotides, β-glucans, mannans, and other bioactive compounds. Dietary yeast-derived products have been reported to affect growth, immune-related responses, oxidative status, and intestinal microbial interactions in aquaculture species (Rodrigues *et al*., 2018; Becerril-Cortés *et al*., 2022; Linh *et al*., 2025). The use of yeast or yeast-based products under BFT conditions also suggests that these materials may influence not only the cultured animal but also the surrounding culture environment (Becerril-Cortés *et al*., 2022; Linh *et al*., 2025). However, most previous studies have used yeast products as dietary ingredients or as components of probiotic formulations. The effects of applying yeast extract directly to BFT culture water on biofloc development, water quality, microbial abundance, and shrimp performance remain insufficiently understood.

Paprika- and red pepper-derived materials are also among the plant-based products investigated in aquaculture. *Capsicum annuum* contains carotenoids and other biologically active compounds that may influence pigmentation, antioxidant defence, and physiological responses. The potential role of plant-derived carotenoids in the nutrition of *P. vannamei* has previously been demonstrated (Ponce-Palafox *et al*., 2006). Although diets containing paprika extract had limited effects on the growth performance of Pacific white shrimp, they were reported to cause changes in haemolymph chemistry, intestinal microbiota, and antioxidant enzyme activities (Erfanifar *et al*., 2024). Paprika-containing diets have also been studied in relation to spermatophore and sperm quality in shrimp, as well as growth and survival in different shrimp species (Braga *et al*., 2013; Díaz Mendoza *et al*., 2020). In addition, red pepper powder has been reported to support antioxidant and immune-related functions in sea urchins (Guo *et al*., 2025). Nevertheless, most of these studies evaluated paprika extract or red pepper powder as a dietary additive. The effects of applying dried sweet red pepper powder directly to BFT culture water have not yet been sufficiently investigated.

It is therefore important to distinguish between dietary supplementation and direct application to culture water. Before being consumed by shrimp, a material added to the culture water may interact with microbial communities, suspended organic matter, and the biofloc matrix. These interactions may alter the nutritional composition of bioflocs, the abundance of culturable bacteria, or important water quality variables. The combined application of yeast extract and dried sweet red pepper powder may also produce

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complementary or interactive responses. However, such effects cannot be predicted solely from the individual properties of these materials and must be evaluated experimentally. This study investigated the effects of yeast extract and dried sweet red pepper powder, applied separately or in combination to the culture water, on *P. vannamei* reared under BFT conditions. Both materials were applied at 0, 1, and 3 mg L^{-1} day^{-1} over a 60-day experimental period using a 3 × 3 factorial design. Treatment effects were evaluated in terms of shrimp growth performance, feed conversion ratio, survival, whole-body proximate composition, and total haemocyte count. Water quality, the proximate composition of the bioflocs, and the total culturable heterotrophic bacterial count in the BFT water were also examined. The study was therefore designed to assess whether yeast extract and dried sweet red pepper powder influenced shrimp performance through direct nutritional contributions, changes induced within the BFT environment, or an interaction between the two materials.

Materials and methods

Experimental design and culture conditions

The study was conducted in the indoor culture facility of the Department of Fisheries and Aquaculture Engineering, Faculty of Agriculture, Ankara University. The 60-day experiment was carried out using Pacific white shrimp (*Penaeus vannamei*) with an initial mean individual weight of approximately 1.50 g. The shrimp were obtained from Anatolian Shrimp Company (Milas, Muğla, Türkiye) and acclimated to laboratory conditions for 10 days before the experiment. The experiment was arranged in a 3×3 factorial design consisting of three application levels each of yeast extract (YE) and dried sweet red pepper powder (SRP). The daily application levels for both materials were 0, 1, and 3 mg L^{-1}. Accordingly, nine treatment groups were established, with each group represented by three independent tanks. Tanks with a nominal capacity of 50 L were filled with 45 L of culture water, and 20 shrimp were stocked in each tank. A total of 540 shrimp distributed among 27 tanks were used in the experiment. Shrimp and treatment groups were randomly assigned to the tanks. The same basal experimental diet was used in all groups, and feeding, aeration, carbon-source addition, and water management were carried out according to a common protocol. The tank was considered the independent experimental unit in all statistical analyses. The use of the tank as the experimental unit under BFT conditions is important for maintaining independence among tanks and for treating individual shrimp samples as subsamples within each tank (Kaya *et al*., 2019, 2020).

Establishment and maintenance of the biofloc system

Artificial sea salt (ReeFlowers Caledonia Sea Salt, İstanbul, Türkiye) and reverse-osmosis-treated tap water were used to prepare the culture water. At the beginning of the experiment, each

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tank was filled with 45 L of reverse-osmosis water, and salinity was adjusted to 22±1 ppt by adding artificial sea salt. After the water temperature reached approximately 28°C, one air stone was placed in each of two corners of every tank, and the system was aerated for 24 h. The BFT system was initiated through natural microbial development. Molasses (Türkiye Şeker, Ankara, Türkiye) was used as the carbon source, while feed and shrimp waste served as nitrogen sources during both the 10-day acclimation and natural biofloc maturation period and the subsequent 60-day experimental period. Carbon addition was adjusted to maintain a target C/N ratio of approximately 15. The amount of molasses required was calculated according to the amount of nitrogen entering the system, assuming that the molasses contained approximately 50% carbon. This approach is commonly used in BFT systems to promote the formation of heterotrophic microbial biomass and facilitate the microbial conversion of nitrogenous compounds (Avnimelech, 1999; Crab *et al*., 2012; Avnimelech, 2012; Emerenciano *et al*., 2017; Kaya, 2025). Continuous aeration was provided to keep the bioflocs suspended in the water column and meet the oxygen requirements of the shrimp. During the acclimation period, the shrimp were placed in the tanks and biofloc development was monitored for 10 days. The treatment period began after the shrimp had fully adapted to the experimental conditions. Water-quality variables and settleable biofloc volume were monitored throughout the 60-day experiment according to the procedures described in Section 2.5. Molasses was added daily throughout the experiment. Carbon and nitrogen inputs originating from YE and SRP were not included in the calculation of the target C/N ratio. Water lost through evaporation was replaced, and approximately 1–2% of the water was exchanged weekly to remove excess suspended solids, based on Imhoff cone measurements. Biofloc volume was measured by allowing a 1-L water sample to settle in an Imhoff cone for 30 min, and the results were recorded as mL L^{-1}. The same water-management protocol was applied to all treatment groups throughout the experiment.

Additives and application levels

Yeast extract (YE; ALFASOL, Katkı Deposu, Türkiye; originating from France according to the supplier) and dried sweet red pepper powder (SRP; *Capsicum annuum*) were applied directly to the culture water. The sweet red pepper powder was prepared from peppers obtained from Çanakkale, Türkiye. After the outer flesh of the peppers had been separated and chopped using a domestic food processor (Krups, Germany), the material was dried at 45°C for 72 h. The dried material was then ground using a Severin KM3872 grinder (Germany), packaged, and stored at −24°C until use. The application doses of both materials were calculated according to their actual weights and the working water volume in each tank. Daily application levels of 1 and 3 mg L^{-1} corresponded to 45 and

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135 mg tank^{-1} day^{-1}, respectively, for each material in tanks containing 45 L of culture water. In the combined-treatment groups, YE and SRP were applied together at their respective assigned doses. The experimental groups and the daily amounts of additives applied per tank are presented in Table 1. The additives were prepared fresh each day and applied to the tanks after the morning feeding. YE and SRP were prepared separately in 100 mL of carrier water. YE was dissolved in the carrier water, whereas SRP was mixed using a handheld battery-operated mixer (Kiwi KCW-1223, China) to obtain a homogeneous suspension. In the combined-treatment groups, both materials were prepared in the same volume of carrier water at their assigned doses and added to the tanks during the same application. An equal volume of carrier water was applied to all groups. The control tanks received the same volume of carrier water without either additive. Thus, the only difference among treatments was the input of YE and SRP. Although paprika- and red pepper-derived materials have previously been used mainly as dietary additives in aquaculture (Ponce-Palafox *et al*., 2006; Erfanifar *et al*., 2024), both materials were applied directly to the BFT culture water in the present study.

Table 1: Experimental groups and daily amounts of additives applied to the culture water.

Group*	YE (mg L^{-1} day^{-1})	SRP (mg L^{-1} day^{-1})	YE (mg tank^{-1} day^{-1})	SRP (mg tank^{-1} day^{-1})
Control	0	0	0	0
YE1	1	0	45	0
YE3	3	0	135	0
SRP1	0	1	0	45
SRP3	0	3	0	135
YE1SRP1	1	1	45	45
YE1SRP3	1	3	45	135
YE3SRP1	3	1	135	45
YE3SRP3	3	3	135	135

*YE: yeast extract; SRP: dried sweet red pepper powder. Amounts per tank were calculated for a working water volume of 45 L. Values represent the amounts added daily.

Feeding and growth performance

The same basal experimental diet was used for all treatment groups throughout the experiment. YE and SRP were applied directly to the culture water and were not incorporated into the diet. The ingredient composition and nutritional characteristics of the experimental diet are presented in Table 2. The experimental diet was formulated according to the nutritional recommendations for shrimp (NRC, 2011). All ingredients were ground to obtain a fine particle size, after which the macro-ingredients and then the micro-ingredients were added to the mixture. The ingredients were mixed thoroughly in a laboratory mixer, and water was added to obtain a dough-like consistency. The mixture was processed using a cold-extrusion pelletiser to produce pellets 1.2 mm in diameter and

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3–4 mm in length. After pelleting, the pellets were heat-treated in a pressurised steam cooker at 1 atmosphere for 20 min to improve their binding properties and water stability. They were subsequently dried at 40°C until a constant weight was reached and stored at +4°C until use. The shrimp were fed three times daily using a modified version of the feeding protocol described by Kaya *et al*. (2020). The daily feeding rate was set at 8% of the initial biomass during the first 15 days, 6% from days 15 to 30, 4% from days 30 to 45, and 3% from days 45 to 60. These rates were adjusted according to the live biomass in each tank and observations of feed consumption. Tank biomass and feeding amounts were reassessed at 15-day intervals, and the amount of feed provided was adjusted when necessary. The amount of feed supplied to each tank and all mortalities were recorded on a tank basis throughout the experiment.

Table 2: Ingredient composition and nutritional characteristics of the basal experimental diet.

Ingredient or nutritional characteristic	Inclusion level or analysed value
Fishmeal[1] (%)	30.0
Soybean meal[2] (%)	12.4
Corn gluten meal[3] (%)	12.4
Wheat flour[4] (%)	35.0
Fish oil[5] (%)	3.4
Soy lecithin[6] (%)	2.2
Vitamin premix[7] (%)	0.8
Mineral premix[7] (%)	0.2
Vitamin C (%)	0.1
Cholesterol[6] (%)	0.5
Binder (guar gum)[8] (%)	3.0
Total (%)	**100.0**
Nutritional composition (% dry matter)	
Crude protein	37.53 ± 0.46
Crude lipid	8.28 ± 0.37
Crude ash	16.28 ± 0.51
Calculated total carbohydrate	37.91
Calculated gross energy (MJ kg^{-1} dry matter)	18.65

[1] Anchovy fishmeal, Sursan Feed Mill Company, Samsun, Türkiye.
[2] Kırcı Soya Company, Balıkesir, Türkiye.
[3] Cargill, İstanbul, Türkiye.
[4] Ipek Wheat Company, Nevşehir, Türkiye.
[5] Anchovy fish oil, Sursan Feed Mill Company, Samsun, Türkiye.
[6] Sigma-Aldrich Chemicals, St. Louis, MO, USA.
[7] DSM Nutritional Products, Türkiye.
[8] Guar gum, Kartal Chemical Company, İstanbul, Türkiye.

The feed ingredients and additives were included at the following levels: 30.0% fishmeal, 12.4% soybean meal, 12.4% corn gluten meal, 35.0% wheat flour, 3.4% fish oil, 2.2% soy lecithin, 0.8% vitamin premix, 0.2% mineral premix, 0.1% vitamin C, 0.5% cholesterol, and 3.0% guar gum. The total inclusion level of the ingredients was adjusted to 100%. The crude protein, crude lipid, and crude ash contents of the diet on a dry-matter basis were 37.53±0.46%, 8.28±0.37%,

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and 16.28±0.51%, respectively. Total carbohydrate content was calculated on a dry-matter basis using the following equation:

Total carbohydrate (%) = 100 − [crude protein (%) + crude lipid (%) + crude ash (%)]

According to this calculation, the total carbohydrate content of the diet was 37.91%. Gross energy was estimated using coefficients of 23.6, 39.5, and 17.2 kJ g^{-1} for protein, lipid, and carbohydrate, respectively. The estimated gross energy content of the diet was therefore 18.65 MJ kg^{-1} dry matter. Nutritional composition values are presented as mean ± standard deviation on a dry-matter basis. At the beginning of the experiment and on day 60, five shrimp were randomly selected from each tank and weighed individually using a RADWAG WLC 0.6/A1/C/2 IO precision balance with an accuracy of 0.01 g (RADWAG, Poland). Weighing was performed before the morning feeding following a fasting period of approximately 13–14 h. The initial and final mean individual weights for each tank were calculated as the arithmetic means of the five shrimp weighed at the respective sampling time. Weight gain, specific growth rate, feed conversion ratio, and survival rate were calculated on a tank basis.

Growth and production indices were calculated using the equations presented below:

$WG\ (g\ shrimp^{-1}) = Wf - Wi$

$SGR\ (\%\ day^{-1}) = 100 \times [ln(Wf) - ln(Wi)] / t$

$FCR = F / \Delta B$

$SR\ (\%) = 100 \times Nf / Ni$

$\Delta B = Bf - Bi$

Wi and Wf are the initial and final mean individual weights (g), respectively; F is the total dry feed supplied per tank (g); ΔB is the increase in tank biomass (g); Ni and Nf are the initial and final numbers of live shrimp, respectively; and t is the experimental duration (days). Bi is the initial tank biomass and Bf is the final tank biomass of shrimp during the experiment.

Water quality and biofloc volume

Water temperature, dissolved oxygen concentration, pH, and salinity were measured daily in each tank before the morning feeding and additive application. Temperature, dissolved oxygen, and pH were measured using a YSI 556 multiparameter water-quality meter (YSI Inc., Yellow Springs, OH, USA). Salinity was determined using an ATC refractometer (China). For chemical analyses, a 50-mL water sample was collected from the middle of the water column of each tank at five-day intervals. Nitrite, nitrate, total ammonia nitrogen, alkalinity, and phosphate concentrations were measured using an Iris Visible Spectrophotometer (HI801-01, Hanna Instruments, USA). Ammonia results were expressed as total ammonia nitrogen (TAN), nitrite as NO_2–N, nitrate as NO_3–N, and phosphate as PO_4–P. Biofloc volume was determined after allowing the water samples to settle in a

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standard Imhoff cone for 30 min. The measured values were recorded as mL L^{-1}. All measurements and analytical results were recorded together with the tank number and sampling date.

Proximate analyses

The nutritional composition of the experimental diet, shrimp whole-body samples, and biofloc was determined using the standard methods described by AOAC (2000). For the shrimp and biofloc analyses, three individual shrimp or three separate samples were collected from each tank and analysed separately while retaining their tank identities. Before analysis, the samples were homogenised and stored at −24°C. Dry-matter content was determined from the difference between sample weights before and after drying. Dry matter was calculated as the percentage ratio of the sample weight remaining after drying to the initial wet sample weight. Crude ash was determined gravimetrically from the inorganic residue remaining after the samples had been incinerated in a muffle furnace. Total nitrogen was determined using the Kjeldahl method, and the resulting nitrogen value was multiplied by 6.25 to calculate crude protein content. Crude lipid content was determined using an ANKOM XT-15 automated solvent extraction system (ANKOM Technology, Macedon, NY, USA). After the petroleum ether solvent had been removed, the recovered extract was weighed and the crude lipid content was calculated. Crude protein, crude lipid, and crude ash were expressed as percentages of dry matter, whereas dry-matter content was expressed as a percentage of wet sample weight. The whole-body composition of the shrimp and the proximate composition of the bioflocs are presented in separate tables. The analytical results from the three individual shrimp or samples collected from each tank were averaged to obtain a single tank value, which was used as the independent observation in the statistical analyses.

Total culturable heterotrophic bacterial count in BFT water

The total culturable heterotrophic bacterial density in the BFT water was determined using a plate-count method adapted from Kaya *et al*. (2019). At the end of the experiment, three separate water samples were collected from each tank in sterile 50-mL glass bottles and analysed separately. Tenfold serial dilutions were prepared from the water samples using sterile physiological saline. A 0.1-mL aliquot of an appropriate dilution was spread onto the surface of previously prepared Plate Count Agar containing 2% NaCl (PCA; Merck, Germany). The plates were incubated under aerobic conditions, initially at 37°C for 48 h and subsequently at 25°C for five days. Colonies were counted at the end of the incubation period, and only plates containing 30–300 colonies were included in the calculations.

Bacterial density was calculated using the following equation: Bacterial density (CFU mL^{-1})=C / (V × d)

C is the number of colonies counted, V is the plated volume (0.1 mL), and d is

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the decimal dilution plated (e.g. 10^{-6}). The results were initially calculated as CFU mL^{-1}. Values from the three water samples collected from each tank were averaged to obtain a single value per tank. The results are presented in the tables on the original scale as $\times 10^6$ CFU mL^{-1}. Before statistical analysis, a $\log_{10}$ transformation was applied to the tank means. The technical samples were treated as subsamples of the tank and were not considered independent experimental units.

Total haemocyte count

Total haemocyte count was determined using a modification of the method described by Söderhäll and Smith (1983). At the end of the experiment, three shrimp were sampled from each tank, and haemolymph was collected separately from each individual. A 100-µL haemolymph sample was withdrawn from the ventral sinus using a sterile 1-mL syringe. The haemolymph was mixed with an equal volume of 10% formalin prepared in 0.45 M NaCl and fixed at room temperature for 10 min. A 10-µL aliquot of the prepared cell suspension was transferred to a haemocytometer chamber and allowed to stand at room temperature for 5 min so that the cells could settle on the counting surface. Haemocytes were then counted in 12 squares under an Olympus CX-23 trinocular microscope at ×40 magnification.

Total haemocyte count was calculated using the following equation:

THC (cells mL^{-1})=(C×D) /Vc

C is the total number of haemocytes counted, D is the dilution factor, and Vc is the total chamber volume counted (mL). In this study, D was 2 because equal volumes of haemolymph and fixative were mixed. The results were expressed as $\times 10^6$ cells mL^{-1} haemolymph. The individual total haemocyte counts of the three shrimp sampled from the same tank were averaged to obtain a single tank value, and the tank was used as the experimental unit in the statistical analyses (Kaya *et al*., 2020; Genc *et al*., 2024a).

Statistical analysis

The tank was considered the independent experimental unit in all statistical analyses, and each treatment was represented by three independent tanks. Initial and final weights were calculated on a tank basis using the mean of the five shrimp weighed from each tank. For total haemocyte count, total heterotrophic bacterial count in BFT water, and proximate-composition measurements, the results from three individual shrimp or samples collected from each tank were averaged to obtain a single value per tank. Results are presented as the mean±standard deviation of three independent tanks. Growth performance, feed conversion ratio, survival rate, total haemocyte count, total culturable heterotrophic bacterial count, and proximate-composition data were analysed using two-way analysis of variance. YE level (0, 1, and 3 mg L^{-1} day^{-1}), SRP level (0, 1, and 3 mg L^{-1} day^{-1}), and their

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interaction were included in the statistical model as fixed effects. The normality of the model residuals was assessed using the Shapiro–Wilk test, and homogeneity of variance was examined using the median-centred Levene test. For the bacterial count data, the arithmetic mean of the three subsamples was first calculated for each tank, after which the tank means were $\log_{10}$-transformed. Statistical analyses of bacterial counts were performed using the transformed data, whereas the results are presented in the tables on the original scale as $\times 10^6$ CFU mL^{-1}. When the interaction between the two factors was not significant but a main effect was significant, the marginal means of the relevant factor, averaged across the levels of the other factor, were compared using Tukey-adjusted pairwise comparisons. Complementary group-level comparisons were also performed for total haemocyte count, total culturable heterotrophic bacterial count, and proximate-composition variables. Pairwise differences among the nine treatment groups were examined using Tukey's HSD test, while each treatment group was compared with the control using a two-sided Dunnett test. All comparisons were performed using tank means. Statistical significance was accepted at $p<0.05$. All statistical analyses were conducted using SPSS version 24.0.

Results

Water quality and biofloc volume

The group means for water temperature, dissolved oxygen, pH, nitrogenous compounds, alkalinity, phosphate, and settleable biofloc volume during the 60-day experiment are presented in Table 3.

Table 3: Water-quality parameters and settleable biofloc volume during the 60-day experiment.

Parameter*	Control	YE1	YE3	SRP1	SRP3	YE1SRP1	YE1SRP3	YE3SRP1	YE3SRP3
Temperature (°C)	27.68 ± 0.63	28.02 ± 0.54	27.94 ± 0.71	27.57 ± 0.61	28.06 ± 0.48	27.55 ± 0.82	28.11 ± 0.73	28.06 ± 0.83	27.55 ± 0.57
Dissolved oxygen (mg L^{-1})	6.57 ± 0.49	6.58 ± 0.61	6.59 ± 0.55	6.54 ± 0.48	6.53 ± 0.59	6.49 ± 0.63	6.56 ± 0.42	6.52 ± 0.51	6.48 ± 0.47
pH	8.04 ± 0.13	8.06 ± 0.15	8.05 ± 0.16	8.02 ± 0.10	8.03 ± 0.17	8.07 ± 0.16	8.01 ± 0.11	8.08 ± 0.14	8.05 ± 0.16
Nitrite (NO_2–N, mg L^{-1})	0.38 ± 0.05	0.37 ± 0.04	0.38 ± 0.07	0.37 ± 0.05	0.38 ± 0.06	0.37 ± 0.06	0.39 ± 0.06	0.39 ± 0.05	0.39 ± 0.07
Nitrate (NO_3–N, mg L^{-1})	11.04 ± 0.37	10.86 ± 0.64	11.09 ± 0.22	11.03 ± 0.31	10.74 ± 0.47	10.83 ± 0.53	10.79 ± 0.48	11.01 ± 0.39	11.02 ± 0.41
Total ammonia nitrogen (mg L^{-1})	0.45 ± 0.03	0.44 ± 0.02	0.45 ± 0.02	0.44 ± 0.03	0.46 ± 0.03	0.44 ± 0.04	0.45 ± 0.02	0.46 ± 0.02	0.44 ± 0.03
Alkalinity (mg $CaCO_3$ L^{-1})	107.17 ± 2.55	108.02 ± 3.12	105.83 ± 2.97	106.46 ± 3.03	107.11 ± 2.70	106.54 ± 2.49	108.08 ± 2.04	107.16 ± 2.18	106.78 ± 2.68
Phosphate (PO_4–P, mg L^{-1})	0.68 ± 0.03	0.68 ± 0.01	0.69 ± 0.04	0.67 ± 0.02	0.69 ± 0.02	0.68 ± 0.02	0.67 ± 0.03	0.69 ± 0.03	0.68 ± 0.04
Biofloc volume (mL L^{-1})	15.11 ± 1.07	14.78 ± 1.21	15.06 ± 1.17	15.02 ± 1.09	14.90 ± 1.24	15.14 ± 1.12	15.07 ± 1.27	14.83 ± 1.25	15.01 ± 1.31

*Values are presented as mean ± standard deviation. YE: yeast extract; SRP: dried sweet red pepper powder. No statistical comparisons among treatment groups are presented in this table.

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Mean water temperature ranged from 27.55 to 28.11 °C, dissolved oxygen concentration from 6.48 to 6.59 mg L^{-1}, and pH from 8.01 to 8.08. The group means for nitrite, nitrate, and total ammonia nitrogen ranged from 0.37 to 0.39, 10.74 to 11.09, and 0.44 to 0.46 mg L^{-1}, respectively. Mean alkalinity ranged from 105.83 to 108.08 mg $CaCO_3$ L^{-1}, while phosphate concentration ranged from 0.67 to 0.69 mg L^{-1}. The mean settleable biofloc volume ranged from 14.78 to 15.14 mL L^{-1}. These ranges represent the treatment-group means and not the lowest and highest individual measurements recorded during the experiment.

Growth performance, feed conversion ratio, and survival

At the beginning of the experiment, mean individual weights ranged from 1.44 to 1.51 g among the treatment groups. The effects of YE, SRP, and their interaction on initial weight were not significant (p>0.05). At the end of the experiment, the lowest mean final weight was recorded in the Control group (7.66±0.22 g), whereas the highest was recorded in the YE3SRP1 group (8.67±0.28 g). Weight gain ranged from 6.16 to 7.17 g $shrimp^{-1}$, and specific growth rate ranged from 2.71 to 2.92% day^{-1} (Table 4).

Table 4: Growth performance, feed conversion ratio, and survival of Penaeus vannamei during the 60-day experiment: treatment-group results and two-way ANOVA.

Group*	IW (g)	FW (g)	WG (g $shrimp^{-1}$)	SGR (% day^{-1})	FCR	SR (%)
Control	1.51 ± 0.02	7.66 ± 0.22	6.16 ± 0.22	2.71 ± 0.04	1.657 ± 0.047	80.00 ± 5.00
YE1	1.51 ± 0.07	7.98 ± 0.39	6.47 ± 0.35	2.78 ± 0.08	1.590 ± 0.020	83.33 ± 7.64
YE3	1.50 ± 0.05	8.03 ± 0.26	6.52 ± 0.24	2.79 ± 0.06	1.580 ± 0.050	86.67 ± 10.41
SRP1	1.50 ± 0.06	7.88 ± 0.28	6.37 ± 0.26	2.76 ± 0.06	1.620 ± 0.026	85.00 ± 8.66
SRP3	1.51 ± 0.07	7.78 ± 0.09	6.28 ± 0.14	2.74 ± 0.09	1.640 ± 0.066	83.33 ± 5.77
YE1SRP1	1.50 ± 0.10	8.12 ± 0.32	6.62 ± 0.33	2.81 ± 0.12	1.590 ± 0.020	85.00 ± 5.00
YE1SRP3	1.44 ± 0.07	8.27 ± 0.58	6.84 ± 0.62	2.91 ± 0.18	1.570 ± 0.026	85.00 ± 5.00
YE3SRP1	1.50 ± 0.05	8.67 ± 0.28	7.17 ± 0.32	2.92 ± 0.11	1.567 ± 0.015	88.33 ± 5.77
YE3SRP3	1.50 ± 0.01	8.64 ± 0.30	7.13 ± 0.29	2.91 ± 0.05	1.543 ± 0.050	88.33 ± 5.77
Two-way ANOVA (p)						
YE	0.6761	0.0015	0.0016	0.0188	0.0021	0.3075
SRP	0.6830	0.0646	0.0579	0.1195	0.4232	0.6633
YE × SRP	0.8226	0.5890	0.5774	0.6828	0.8511	0.9927

***** Values are presented as the mean ± standard deviation of three independent tanks (n = 3). IW: initial weight; FW: final weight; WG: weight gain; SGR: specific growth rate; FCR: feed conversion ratio; SR: survival rate. Calculations were performed using unrounded tank values. Significant p-values are shown in bold.

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Two-way ANOVA showed a significant main effect of YE on final weight, weight gain, and specific growth rate (p=0.0015, p = 0.0016, and p=0.0188, respectively). Neither the main effect of SRP nor the YE × SRP interaction was significant for these variables. Based on Tukey comparisons of the marginal means calculated across SRP levels, final weight, weight gain, and specific growth rate were significantly higher at YE3 than at YE0. The YE1 level did not differ significantly from either YE0 or YE3 for these variables (Table 5). Mean feed conversion ratio ranged from 1.543 to 1.657 among the treatment groups. YE had a significant main effect on FCR (p=0.0021), whereas the effects of SRP and the YE×SRP interaction were not significant. Comparison of the marginal means showed that FCR values at YE1 and YE3 were significantly lower than that at YE0, with no significant difference between YE1 and YE3. Survival was 80.00 ± 5.00% in the Control group and 88.33 ± 5.77% in both the YE3SRP1 and YE3SRP3 groups. YE, SRP, and their interaction had no significant effects on survival (p>0.05).

Table 5: Growth performance and feed conversion ratio of Penaeus vannamei during the 60-day experiment: marginal means for yeast extract levels

YE level (mg L^{-1} day^{-1})	FW (g)	WG (g $shrimp^{-1}$)	SGR (% day^{-1})	FCR
0	7.77^{b}	6.27^{b}	2.74^{b}	1.639^{a}
1	8.12^{ab}	6.64^{ab}	2.84^{ab}	1.583^{b}
3	8.45^{a}	6.94^{a}	2.87^{a}	1.563^{b}

*Marginal means for each YE level were calculated across the three SRP levels. YE0 includes the Control, SRP1, and SRP3 groups. Within a column, marginal means without a common superscript letter differ significantly according to Tukey-adjusted comparisons (p<0.05). The superscript letters do not represent pairwise comparisons among the nine treatment groups presented in Table 4. FW: final weight; WG: weight gain; SGR: specific growth rate; FCR: feed conversion ratio.

Total haemocyte count in shrimp haemolymph

The group means for total haemocyte count in shrimp haemolymph ranged from 2.62 to 3.62×10^{6} cells mL^{-1} (Table 6). The lowest mean was recorded in the Control group, while the highest was observed in the YE3SRP3 group. The main effects of YE (p=0.3944) and SRP (p=0.8117), as well as their interaction (p=0.2015), were not significant. Complementary Tukey comparisons showed no significant differences among the nine treatment groups. Similarly, two-sided Dunnett comparisons between each treatment and the Control group revealed no significant differences (p>0.05).

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Table 6: Total haemocyte count in the haemolymph of Penaeus vannamei subjected to different yeast extract and sweet red pepper powder treatments

Group*	Total haemocyte count (×10⁶ cells mL⁻¹)
Control	2.62 ± 0.17
YE1	3.51 ± 0.43
YE3	3.54 ± 0.73
SRP1	3.46 ± 0.46
SRP3	3.27 ± 0.34
YE1SRP1	3.49 ± 0.46
YE1SRP3	3.21 ± 0.45
YE3SRP1	3.09 ± 0.85
YE3SRP3	3.62 ± 0.33
Two-way ANOVA (p)	
YE	0.3944
SRP	0.8117
YE × SRP	0.2015

*Values are presented as the mean ± standard deviation of three independent tanks (n = 3). Each tank value was calculated as the mean of the total haemocyte counts obtained from three individual shrimp. YE: yeast extract; SRP: dried sweet red pepper powder. Neither Tukey nor two-sided Dunnett comparisons showed significant differences (p>0.05).

Total heterotrophic bacterial count in BFT water

The group means for total culturable heterotrophic bacterial density in the BFT water ranged from 7.94 to 9.38 × 10^6 CFU mL^{-1} (Table 7). The lowest mean was recorded in the Control group at 7.94±0.68×10^6 CFU mL^{-1}, whereas the highest was recorded in the YE3SRP3 group at 9.38±0.80×10^6 CFU mL^{-1}. Analysis of the $\log_{10}$-transformed tank means showed that the main effects of YE (p=0.2110) and SRP (p=0.4616), as well as their interaction (p=0.9950), were not significant. Complementary Tukey comparisons revealed no significant differences among the treatment groups, and two-sided Dunnett comparisons showed no significant differences between any treatment and the Control group (p > 0.05).

Table 7: Total culturable heterotrophic bacterial count in BFT water under different yeast extract and sweet red pepper powder treatments

Group*	Total heterotrophic bacterial count (×10⁶ CFU mL⁻¹)
Control	7.94 ± 0.68
YE1	8.58 ± 0.99
YE3	8.77 ± 0.97
SRP1	8.14 ± 0.93
SRP3	8.63 ± 0.77
YE1SRP1	8.47 ± 0.29
YE1SRP3	8.86 ± 0.99
YE3SRP1	9.05 ± 1.54
YE3SRP3	9.38 ± 0.80
Two-way ANOVA (p)	
YE	0.2110
SRP	0.4616
YE × SRP	0.9950

*Values are presented as the mean ± standard deviation of three independent tanks (n = 3). Each tank value represents the arithmetic mean of the bacterial densities determined in three separate BFT water samples. Results are presented on the original scale, whereas statistical analyses were performed using $\log_{10}$-transformed tank means. YE: yeast extract; SRP: dried sweet red pepper powder. Neither Tukey nor two-sided Dunnett comparisons showed significant differences (p>0.05).

Whole-body proximate composition of shrimp

The group means for whole-body crude protein content ranged from 68.03 to 71.68% on a dry-matter basis. The

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lowest mean was recorded in the Control group, while the highest was observed in the YE3SRP3 group. Crude lipid content ranged from 2.34 to 2.66%, and crude ash content ranged from 10.93 to 11.14%. Mean dry-matter content, expressed on a wet-weight basis, ranged from 23.62 to 24.89% (Table 8). Two-way ANOVA showed no significant effects of YE, SRP, or their interaction on whole-body crude protein, crude lipid, crude ash, or dry-matter content (p>0.05). Complementary Tukey comparisons showed no significant differences among the treatment groups, and two-sided Dunnett comparisons showed no significant differences between any treatment and the Control group.

Proximate composition of bioflocs

The crude protein content of the biofloc samples ranged from 26.67 to 31.27%, crude lipid content from 0.35 to 0.46%, and crude ash content from 23.36 to 24.86% (Table 9). Dry-matter content, expressed on a wet-weight basis, ranged from 22.18 to 22.97%. The lowest crude protein value was recorded in the Control group (26.67±2.21%), whereas the highest was recorded in the YE3 group (31.27±1.42%). According to the two-way ANOVA results, YE had no significant main effect on crude protein (p=0.205), crude lipid (p=0.248), crude ash (p=0.198), or dry-matter content (p=0.078). Similarly, SRP had no significant effect on crude protein (p=0.966), crude lipid (p=0.462), crude ash (p=0.668), or dry-matter content (p=0.832). The YE×SRP interaction was also not significant for crude protein (p=0.666), crude lipid (p=0.958), crude ash (p=0.995), or dry-matter content (p=0.456). Complementary Tukey and two-sided Dunnett comparisons also showed no significant differences among the treatment groups or between any treatment and the Control group (p>0.05).

Table 8: Whole-body proximate composition of Penaeus vannamei subjected to different yeast extract and sweet red pepper powder treatments.

Group*	Crude protein (% DM)	Crude lipid (% DM)	Crude ash (% DM)	Dry matter (% wet weight)
Control	68.03 ± 2.53	2.34 ± 0.17	11.10 ± 0.22	24.42 ± 1.67
YE1	70.29 ± 0.66	2.56 ± 0.04	10.95 ± 0.26	23.85 ± 0.64
YE3	70.16 ± 2.85	2.65 ± 0.27	11.08 ± 0.12	24.01 ± 0.29
SRP1	69.41 ± 2.08	2.53 ± 0.18	10.99 ± 0.36	24.74 ± 0.92
SRP3	69.76 ± 1.78	2.47 ± 0.10	10.93 ± 0.31	24.32 ± 0.56
YE1SRP1	71.48 ± 1.56	2.57 ± 0.17	10.96 ± 0.16	23.62 ± 1.20
YE1SRP3	69.86 ± 1.32	2.66 ± 0.22	11.05 ± 0.16	24.62 ± 1.34
YE3SRP1	70.69 ± 0.22	2.48 ± 0.06	11.14 ± 0.08	24.89 ± 1.27
YE3SRP3	71.68 ± 1.22	2.56 ± 0.19	11.01 ± 0.20	24.30 ± 0.55
Two-way ANOVA (p)				
YE	0.1008	0.1907	0.6678	0.6128
SRP	0.4078	0.8386	0.8935	0.7480
YE × SRP	0.6982	0.4633	0.8324	0.6750

*Values are presented as the mean ± standard deviation of three independent tanks (n = 3). Each tank value was calculated as the mean of whole-body analyses performed on three individual shrimp. DM: dry matter; YE: yeast extract; SRP: dried sweet red pepper powder. Crude protein, crude lipid, and crude ash are expressed on a dry-matter basis, whereas dry-matter content is expressed as a percentage of wet sample weight. Neither Tukey nor two-sided Dunnett comparisons showed significant differences (p>0.05).

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Table 9: Proximate composition of bioflocs under different yeast extract and dried sweet red pepper powder treatments and the results of two-way ANOVA

Group*	Crude protein (% DM)	Crude lipid (% DM)	Crude ash (% DM)	Dry matter (% wet weight)
Control	26.67 ± 2.21	0.37 ± 0.05	23.64 ± 0.91	22.43 ± 0.39
YE1	30.49 ± 3.95	0.43 ± 0.11	24.47 ± 0.49	22.58 ± 0.45
YE3	31.27 ± 1.42	0.46 ± 0.02	24.33 ± 0.60	22.73 ± 0.45
SRP1	28.89 ± 3.84	0.35 ± 0.04	23.36 ± 1.15	22.45 ± 0.34
SRP3	28.43 ± 2.40	0.36 ± 0.10	23.89 ± 0.21	22.18 ± 0.05
YE1SRP1	29.31 ± 3.05	0.40 ± 0.03	24.39 ± 0.55	22.90 ± 0.51
YE1SRP3	28.71 ± 3.17	0.38 ± 0.11	24.58 ± 0.22	22.93 ± 0.66
YE3SRP1	29.42 ± 1.17	0.39 ± 0.04	24.19 ± 1.48	22.45 ± 0.28
YE3SRP3	30.36 ± 1.55	0.41 ± 0.06	24.86 ± 2.33	22.97 ± 0.42
Two-way ANOVA (p)				
YE	0.205	0.248	0.198	0.078
SRP	0.966	0.462	0.668	0.832
YE × SRP	0.666	0.958	0.995	0.456

*Values are presented as the mean ± standard deviation of three independent tanks (n = 3). Each tank value was calculated as the mean of analyses performed on three separate biofloc samples collected from that tank. DM: dry matter; YE: yeast extract; SRP: dried sweet red pepper powder. Crude protein, crude lipid, and crude ash are expressed on a dry-matter basis, whereas dry-matter content is expressed as a percentage of wet sample weight. Neither Tukey nor two-sided Dunnett comparisons showed significant differences ($p > 0.05$).

Discussion

This study investigated the effects of the direct daily application of yeast extract (YE) and dried sweet red pepper powder (SRP) to BFT water on *Penaeus vannamei*. The results showed that YE improved final weight, weight gain, specific growth rate, and feed conversion ratio. In contrast, neither SRP nor the YE×SRP interaction had a significant effect on growth performance. Total haemocyte count, culturable heterotrophic bacterial density, shrimp whole-body proximate composition, and the basic proximate composition of the bioflocs were also unaffected by the treatments.

Effects of yeast extract on growth performance and feed utilisation

In the present study, increasing YE levels improved the final weight, weight gain, and specific growth rate of *P. vannamei* and reduced the feed conversion ratio. These findings are generally consistent with previous studies reporting that yeast-derived products may improve growth performance and feed utilisation in aquatic species. Dietary supplementation with yeast-derived mannoproteins has also been associated with the production performance of *P. vannamei* reared under BFT conditions (Rodrigues *et al*., 2018). Similarly, studies on dietary protein optimisation and mannan oligosaccharide supplementation in brown shrimp reared under BFT conditions have shown that growth and production performance may be influenced by interactions between the BFT environment and dietary components (Genc *et al*., 2024a,b). However, the additives in those studies were incorporated into the diet, whereas YE was applied directly to the culture

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water in the present study. This difference suggests that the observed growth responses may have been associated with changes in dissolved organic matter, microbial processes, or nutrient utilisation within the biofloc environment rather than solely with the direct ingestion of the additive.

Yeast extract contains proteins, peptides, nucleotides, β-glucans, mannans, and various soluble organic compounds and may therefore function as a material capable of influencing nutrient utilisation and microbial processes in the culture environment (Hassaan *et al*., 2018; Becerril-Cortés *et al*., 2022). Nevertheless, most previous studies have evaluated yeast-derived products as feed ingredients or dietary additives. In the present study, YE was added daily to the BFT water. This application method requires consideration of both the direct nutritional contribution of YE and its possible indirect effects through the culture environment. In BFT systems, uneaten feed, faeces, and other organic inputs can be converted into microbial biomass through controlled carbon addition. This biomass may then be partly consumed by shrimp and serve as a supplementary food source (Crab *et al*., 2012; Emerenciano *et al*., 2017). Carbon-source and organic-matter management are key factors affecting biofloc formation, microbial nutrient cycling, and water quality in BFT systems. The management of carbon input and the C/N ratio is particularly important for promoting heterotrophic microbial biomass and controlling nitrogenous compounds (Avnimelech, 1999; Crab *et al*., 2012; Emerenciano *et al*., 2017). Kaya *et al*. (2020) also demonstrated that the combined management of feeding, biofloc development, and tank conditions is important for growth performance in shrimp culture.

Genc *et al*. (2024a) reported that dietary protein level under BFT conditions was associated with growth, digestive enzyme activity, non-specific immunity, and intestinal microbiota in *F. aztecus*. The effects of functional additives such as mannan oligosaccharides on production performance in BFT systems have also been reported (Genc *et al*., 2024b). Similarly, mannoprotein supplementation has been associated with the production performance of *P. vannamei* under BFT conditions (Rodrigues *et al*., 2018). Together, these findings support the view that the effects of feed components or functional additives in BFT systems should be evaluated not only in relation to nutrients directly consumed by shrimp but also in connection with microbial and nutritional processes in the culture environment. Within this framework, YE may have supplied additional soluble organic and nitrogenous compounds to the BFT environment in the present study. These inputs may have affected the availability of nutrients in the bioflocs or the ability of shrimp to utilise biofloc-derived nutrients without producing detectable changes in biofloc volume or basic proximate composition. Indeed, neither biofloc volume nor the

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protein, lipid, ash, and dry-matter contents of the bioflocs differed significantly among the treatments. Therefore, the positive effect of YE on growth cannot be explained solely by an increase in biofloc quantity. The lower FCR values recorded at the YE levels indicate that the increase in growth was accompanied by improved feed utilisation. This finding suggests that YE may have helped shrimp use the available feed and biofloc resources more efficiently. However, the improvement in FCR should not be considered direct evidence of enhanced digestive capacity. Under BFT conditions, feed intake, biofloc consumption, microbial protein contribution, and energy-use pathways may act together. Previous studies have also shown that dietary components and functional additives can influence growth and feed-utilisation indices under BFT conditions (Genc *et al*., 2024a,b). However, those studies were based on dietary supplementation, whereas YE was added to the culture water in the present study. The observed FCR response may therefore have been related to indirect effects on available microbial nutrients or nutrient-utilisation processes within the BFT environment rather than solely to the direct nutritional contribution of YE. The absence of a clear difference in growth indices between the YE1 and YE3 levels suggests that the lower YE dose may also have been effective within the tested range. In other words, the growth response did not increase in a fully linear manner with increasing YE dose. This finding suggests that functional materials may not provide an additional growth advantage beyond a certain level when applied to a BFT environment. Future studies should therefore examine not only higher doses but also the longer-term application of lower YE doses.

Effects of dried sweet red pepper powder and the YE × SRP interaction

The application of SRP had no significant effect on shrimp growth performance, feed conversion ratio, or survival. This finding agrees with previous evidence indicating that paprika- and red pepper-derived products do not always produce a direct improvement in the growth of aquatic animals. Erfanifar *et al*. (2024) reported that dietary paprika extract had limited effects on the growth performance of *P. vannamei*, although changes were observed in haemolymph chemistry, intestinal microbiota, and antioxidant enzyme activities. The biological effects of paprika- and red pepper-derived materials are generally associated with carotenoids, phenolic compounds, and other plant-derived bioactive components. Plant-derived carotenoids can be used in *P. vannamei* diets and may contribute to pigmentation and antioxidant defence (Ponce-Palafox *et al*., 2006). Paprika-supplemented diets have also been associated with spermatophore and sperm quality in pink shrimp and with growth and survival in freshwater shrimp (Braga *et al*., 2013; Díaz Mendoza *et al*., 2020). Guo *et al*. (2025) further reported that red pepper

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powder may support certain antioxidant and immune-related functions in sea urchins. However, paprika and red pepper materials were administered through the diet in most of these studies. In contrast, SRP was applied directly to the culture water in the present study. Therefore, carotenoids and other bioactive compounds delivered through the diet may not produce the same effects when dried pepper powder is applied directly to water. A proportion of the SRP may have remained undissolved, become associated with the biofloc matrix, or accumulated along the sides or bottom of the tanks. These processes may have limited the availability of its bioactive components to the shrimp and, consequently, reduced its biological effect. The combined application of YE and SRP also produced no significant interaction for the growth variables. Although some combined-treatment groups showed numerically higher final weights and weight gains, these numerical differences do not demonstrate a synergistic relationship between YE and SRP. The non-significant interaction term indicates that there was no statistical evidence that SRP enhanced the growth response associated with YE. Thus, the presence of biologically active compounds in both materials does not necessarily mean that their combined application will produce complementary or synergistic effects.

Water quality, biofloc volume, and heterotrophic bacteria

Water temperature, dissolved oxygen, pH, nitrite, nitrate, total ammonia nitrogen, alkalinity, and phosphate remained within narrow ranges among the treatment groups during the experiment. Biofloc volume was also similar across treatments. These observations indicate that the application of YE and SRP did not cause a marked disturbance in the overall environmental conditions of the BFT system. Water quality in BFT systems is shaped by interactions among feed loading, carbon source, stocking density, aeration, and microbial biomass (Crab *et al*., 2012; Emerenciano *et al*., 2017; El-Sayed, 2021). Carbon supplementation plays a central role in the formation of heterotrophic microbial biomass and the control of nitrogenous compounds (Avnimelech, 1999; Crab *et al*., 2012; Emerenciano *et al*., 2017; Kaya *et al*., 2019). Previous BFT studies have also shown that shrimp performance should be evaluated in relation to both feed or functional additives and the microbial and physicochemical conditions of the culture environment (Rodrigues *et al*., 2018; Kaya *et al*., 2020; Genc *et al*., 2024a,b). BFT conditions have previously been associated with the intestinal microbiota of shrimp (Hostins *et al*., 2017). In the present study, the total culturable heterotrophic bacterial density in the BFT water ranged from 7.94 to 9.38×10^{6} CFU mL^{-1}, with no significant effects of YE, SRP, or their interaction. These results indicate that the tested application levels did not alter the density of culturable aerobic heterotrophic bacteria. The

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improvement in growth performance following YE application, despite the absence of a change in bacterial count, suggests that the growth response cannot be explained solely by an increase in total culturable bacterial density. However, culturable bacterial counts represent only a limited fraction of the BFT microbiota because the method detects only bacteria capable of growing under the selected culture-medium and incubation conditions. Therefore, similar bacterial densities do not necessarily indicate that the treatments had no effect on microbial community composition. In BFT systems, microbial community structure may change without a corresponding change in total bacterial density. The present findings should therefore be interpreted specifically as evidence that the treatments did not alter the total density of culturable heterotrophic bacteria.

Total haemocyte count

YE, SRP, and their interaction had no significant effect on total haemocyte count. Total haemocyte count is commonly used as a basic indicator of cellular immune status in shrimp. Functional feed additives have been reported to influence shrimp immune responses under BFT conditions (Hostins *et al*., 2017; Rodrigues *et al*., 2018; Hussain *et al*., 2021; Genc *et al*., 2024a). However, the direction and magnitude of an immune response may vary according to the chemical characteristics of the additive, application route, dose, environmental stress level, and prevailing microbial conditions. Erfanifar *et al*. (2024) showed that paprika extract may influence haemolymph chemistry and antioxidant enzyme activities in *P. vannamei*, although these responses do not necessarily occur in parallel with changes in growth. In the present study, the absence of a significant difference in THC indicates that the positive growth response associated with YE was not accompanied by an increase in total haemocyte count. Growth and THC responses may therefore vary independently. The generally suitable and comparable water-quality conditions among the treatment groups may also have limited the occurrence of marked stress-related or immune responses. However, because immune assessment was limited to total haemocyte count, the present findings do not exclude possible effects on other cellular, humoral, or antioxidant indicators.

Proximate composition of shrimp and bioflocs

The whole-body crude protein, crude lipid, crude ash, and dry-matter contents of the shrimp were not significantly affected by the treatments. Thus, although YE improved growth performance, it did not produce a detectable change in basic whole-body composition. The absence of parallel changes in growth and whole-body protein or lipid content suggests that YE may have affected the utilisation of available nutrients rather than substantially altering their relative deposition in the shrimp body. The

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common basal diet used in all groups may have been a major factor determining shrimp body composition. Feeding and culture conditions were also maintained under a common protocol across treatments. Therefore, the addition of YE or SRP to the culture water may not have provided a sufficient contribution to alter basic nutrient intake or the balance of protein and lipid deposition in shrimp tissues. The crude protein, crude lipid, crude ash, and dry-matter contents of the bioflocs also did not differ significantly among the treatment groups. Although crude protein values were numerically higher in some YE groups, this trend was not statistically supported. The chemical composition of bioflocs is determined not only by the amount of additive applied each day but also by uneaten feed, faecal matter, microbial growth, mineral precipitation, and the movement and accumulation of suspended solids within the tanks (Crab *et al*., 2012; Emerenciano *et al*., 2017). Therefore, although YE improved shrimp growth, it did not produce a statistically significant increase in the crude protein content of the bioflocs.

Study limitations

This study evaluated only three application levels of YE and three levels of SRP over a 60-day experimental period. The findings should therefore be interpreted within the tested dose range and the specific BFT conditions used in this study. Different doses, longer application periods, or different stocking densities may produce different responses. YE and SRP were applied directly to the culture water. Consequently, the actual amounts ingested by the shrimp could not be determined. The dissolution, suspension, association with the biofloc matrix, and possible accumulation of these materials on the tank bottom were not evaluated separately. These factors may have influenced their availability to the shrimp and their effects within the BFT environment. The microbial assessment was limited to the total culturable heterotrophic bacterial count, which represents only the fraction of the microbial community able to grow under the selected culture and incubation conditions. Taxonomic composition, microbial diversity, and changes in specific beneficial or potentially harmful microbial groups were not examined using molecular methods. Therefore, the absence of a significant difference in culturable bacterial density should not be interpreted as evidence that the treatments had no effect on microbial community structure. Similarly, immune assessment was limited to total haemocyte count, while the nutritional evaluation of the bioflocs included only crude protein, crude lipid, crude ash, and dry-matter content. Future studies should compare a wider range of YE and SRP doses and directly examine dietary administration and waterborne application. Microbiota composition, digestive and immune indicators, biofloc amino acid profiles, nutrient digestibility, and the distribution and uptake of the applied materials should also be evaluated.

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Conclusion

This study evaluated the effects of applying YE and SRP directly to BFT culture water during the rearing of *Penaeus vannamei*. YE improved final weight, weight gain, specific growth rate, and feed conversion ratio. These improvements occurred while water-quality variables and biofloc volume remained within narrow ranges among treatments, with no significant changes in total culturable heterotrophic bacterial density or the basic whole-body composition of the shrimp. SRP, whether applied alone or in combination with YE, had no significant effect on growth performance, survival, total haemocyte count, bacterial density, shrimp whole-body proximate composition, or biofloc proximate composition. No significant interaction between YE and SRP was detected. Overall, YE may be considered a promising water-applied functional material for supporting the growth and feed utilisation of *P. vannamei* under BFT conditions. In contrast, the direct application of SRP to BFT water provided no additional production benefit at the tested levels. Further studies are required to clarify the mechanisms underlying the YE response and to evaluate different doses and application periods together with microbial, nutritional, digestive, and immune indicators.

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