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Research Article

Comparison of the effect of three distinct diets on the growth, feed efficiency, and body color of whiteleg shrimp (*Litopenaeus vannamei*)

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Abstract

A 60-day feeding trial under composite conditions was conducted to compare the effect of three different treatments of a diet containing 55.50% Fishmeal (FM), a diet with 45.50% FM and 10% Spirulina algae (SP), and a diet with 35.50% FM and 20% *Leucaena leucocephala* powder (LLP) on the growth, feed efficiency, and flesh color of whiteleg shrimp (*Litopenaeus vannamei*). The analysis results of Duncan's Multiple Range *post-hoc* test indicated that survival rate (SR), daily weight gain (DWG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), and protein retention (PR) were significantly improved in the diets containing 10% SP and 20% LLP groups compared to the FM only diet (p<0.05). Noticeably, although the FCR in the treatment of 20% LLP was significantly lower than the treatment of 10% SP and FM, the parameters of PER and PR showed significantly higher performance in 20% LPP treatment in comparison with 10% SP and FM only. Coloration assessments by a sensory method showed that, in their raw form, shrimp fed 10% SP exhibited a more greenish hue, followed by those fed 20% LLP, and then the FM. However, in cooked shrimps, the 20% LLP group displayed the most desirable coloration. A 21-day trial with smaller whiteleg shrimp (0.45 g) confirmed that 20% LLP supplementation positively influenced body color compared to 10% SP and FM-only diets. This study shows that partial replacement of FM with plant ingredients improves shrimp color, increases production sustainability, and enhances primary productivity efficiency.

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Introduction

The whiteleg shrimp (*Litopenaeus vannamei* Boone, 1931) has been the predominant species farmed in Vietnam's aquaculture industry and has brought great economic value; hence, the application of modern farming technologies is used to achieve high-density cultivation. It is well-acknowledged that the reddish color of shrimp is associated with a high-quality product and is favored by consumers (Tume *et al.*, 2009; Parisenti *et al.*, 2011). However, a prevalent issue in the industry is the tendency for cultivated shrimps to exhibit pale body coloring, consequently diminishing their market value. This issue is related to commercial shrimp feeds used in shrimp farming, which often fail to provide sufficient pigmentation, resulting in lighter-colored shrimps with reduced commercial appeal (Yu *et al.*, 2003; Bernal Rodríguez *et al.*, 2017). Farmers have consequently been exploring various carotenoid sources to enhance the coloration of the shrimp cultivated in industrial shrimp farming. Previous studies have identified potential plant-based and synthetic carotenoids in sources like pumpkin, cartilaginous seaweed, Spirulina, and astaxanthin, which could address the issue of paleness in cultivated animals (Cruz-Suárez *et al.*, 1994; Bernal Rodríguez *et al.*, 2017; Manullang *et al.*, 2024). However, these sources have yet to achieve widespread commercial application due to their limitations in achieving high commercial value and practical effectiveness.

Fishmeal (FM) made from wild-caught fish is used as a major dietary protein source in animal diets. In the last few years, the use of FM in the aquaculture sector has increased consistently, rising from 63% in 2009 to around 70% in the period between 2010 and 2016, and then rising further to 78% from 2017 to 2019 (EUMOFA, 2021). Noticeably, the production of FM from pelagic fish is a significant contributor to over-fishing; consequently, a limited amount of FM will be available in the future (Olsen and Hasan, 2012). Overfishing also has negative impacts on the biodiversity of wild fish, ecosystems, and the coastal communities that depend on fisheries. As a result, recent years have seen a growing use of alternative aquafeed ingredients, such as microalgae, seaweeds, yeasts, insects, genetically modified crops, and popular indigenous plants, to replace fishmeal and ensure the sustainability of aquaculture (Majluf *et al.*, 2024; Hussain *et al.*, 2024; Dhar *et al.*, 2024).

Leucaena leucocephala (Lam.) De Wit. (White leadtree) is an endemic and fast-growing leguminous plant naturalized throughout the tropics, including the arid coastal regions of Vietnam. This species has a high protein content (*ca* 30%), along with essential amino acids, vitamins, minerals, carotene, and other antioxidants (Sethi and Kulkani, 1995; Chalaune *et al.*, 2022). This has prompted research into its potential as an alternative feed source in aquaculture. Previous studies suggested that leaf meal of the white leadtree can substitute for up to 25% of conventional feed components such as FM and soybean meal (Sotolu, 2008; Mamat *et al.*, 2017; Lan and To, 2023). Due to its high nutrient content and natural pigments, *L. leucocephala* shows considerable promise as an effective source for improving shrimp

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coloration while maintaining nutritional value.

Arthrospira platensis microalgae (a filamentous and helical cyanobacterium) are widely utilized as a dietary supplement for both humans and animals because of benefits such as improved animal growth, anti-microbial effects, antioxidant effects, and immuno-stimulatory effects (Gauveia *et al.*, 2008; Priyanka *et al.*, 2023; Waheed *et al.*, 2024). Spirulina, including *Arthrospira platensis* has high protein content (50-60%) along with essential fatty acids, vitamins, phytopigments (such as carotenoids, allophycocyanin, C-phycocyanin, and phycoerythrin), essential amino acids, minerals, and bioactive components, and is also easy to digest (Spolaore *et al.*, 2006; Rahim *et al.*, 2021; Priyanka *et al.*, 2023). These attributes make Spirulina an excellent supplement for enhancing nutrition, providing carotenoids, and boosting the health and fertility of animals (Kohal *et al.*, 2018; Sujad and Manohar, 2021; Li *et al.*, 2022).

In related research focused on substituting traditional feed ingredients, Lan and To (2023) reported that *Leucaena* leaf powder could replace up to 20% of FM in diets for whiteleg shrimp without compromising growth, feed efficiency, survival rates, or digestive enzyme activity. Similarly, Zidan *et al.* (2021) found that incorporating 10% algae powder from Spirulina into the diet of whiteleg shrimp yielded optimal results in growth performance. Both *L. leucocephala* and Spirulina hold significant potential as sustainable feed sources that can enhance the growth and coloration of shrimp while reducing feed costs and increasing the commercial value of aquaculture products. Furthermore, *Leucaena* is locally available and cheap; in comparison, commercial powder of Spirulina (*Arthrospira* sp.) is high in cost. However, no previous studies have focused on both the efficiency of *L. leucocephala* as a natural feed source in aquaculture sustainability and its ability to produce the desired shrimp color. Consequently, comparing the effects of incorporating *L. leucocephala* and Spirulina into diets on the growth, feed efficiency, and overall appearance of whiteleg shrimp is necessary to establish the positive effects of popular native plants such as *L. leucocephala* in shrimp culture and increase aquaculture sustainability. The findings of this study could contribute to developing types of aquafeed that are both more sustainable and have a higher economic efficiency in intensive shrimp farming.

Materials and methods

The study on the whiteleg shrimp (*Litopenaeus vannamei*) was approved by the Science and Education Council of Tra Vinh University, Vietnam, regarding the use of animals in research (Decision No. 312/QĐ-HĐKH&ĐT).

Material preparation

Leucaena leucocephala powder (LLP) was prepared using the method described by Lan & To (2023). Particularly, *L. leucocephala* leaves were collected from the rural area of Tra Vinh Province, Vietnam. The leaves were collected from the trees 2.5 m tall and 5 cm in bole diameter. The collected leaves were soaked in water (for 24 h) and dried at 50^{o}C in a laboratory drying oven (for 72 h), then

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ground into powder, and stored at 4°C until use. Powered Spirulina algae (SP) were obtained from a commercial source (Hapu Natural Co. Ltd., Ho Chi Minh City, Vietnam).

Feed preparation

Fishmeal (FM) was produced from shredded fish obtained from Dinh An Fishing Port in Tra Vinh, Vietnam. The fish were dried and finely ground using industrial machinery. The additional ingredients used in the feed formulation included a hydrolyzed protein (Vemedim Co., Can Tho, Vietnam), soy protein (DOMOSAN Manufacturing and Trading Co. Ltd., Ho Chi Minh City, Vietnam), soybean flour, bran, retail cooking oil, and a bio-premix (Bio-Pharmachemie Co., Ho Chi Minh City, Vietnam). The feed was formulated to contain *ca.* 45% protein and consisted of three variations: 1) FM (55.50% of total feed amount); 2) 10% Spirulina algae powder (containing 45.50% FM and 10% algae powder), and 3) 20% *Leucaena leucocephala* powder (containing 35.50% FM and 20% *L. leucocephala* powder) as detailed in Table 1. Compositions were calculated based on the percentage of the total feed amount by weight. It should be noted that due to the lower protein content of LLP treatment in comparison with treatments of 10% SP and FM only, a higher percentage of soybean and soy protein isolate and consequent reduction in the percentage of rice bran was required in the 20% LLP diet in order to balance protein contents across the three diets. The ingredients were mixed in the following order: fine powders, oil, nutrient mixture, and finally water to make a slurry mixture. The mixture was then extruded into pellets using a grain-size machine set to 2 mm. The pellets were dried in natural sunlight, avoiding midday exposure, and stored in plastic bags under room conditions (*ca.* 25-30ºC).

Table 1: Ingredients and proximate compositions of three different diets (% dry weight).

Ingredients	The composition percentages of three different diets		
	Diet 1 (FM)	Diet 2 (10% SP)	Diet 3 (20% LLP)
Fishmeal	55.50	45.50	35.50
Soybean	9.25	9.25	13.44
Soy protein Isolate	10.75	10.75	16.56
Rice bran	15.00	15.00	5.00
L. leucocephala powder (LLP)	-	-	20.00
Spirulina algae (SP)	-	10.00	-
Squid oil	3.00	3.00	3.00
Vitamin mineral premix*	2.00	2.00	2.00
Refined flour	3.00	3.00	3.00
Hydrolysed protein	1.50	1.50	1.50
Total	100.00	100.00	100.00
Proximate composition			
Dry matter	93.50	93.20	93.40
Crude protein	45.60	46.60	45.30
Crude lipid	6.05	5.52	5.68
Ash	13.00	13.20	12.90
NFE	34.60	34.90	35.70
Energy (KJ/g)	19.30	19.30	19.20

* Premix: VitE: 2.700 mg, VitB1: 450 mg, VitA: 270.000 IU, VitD: 135.000 IU, VitB2:360 mg, VitB6: 360 mg, VitK3: 225 mg, Folic acid: 135 mg, Choline Chloride: 4.500, Taurine: 1.800 mg, Calcium Pantothenate: 1.250 mg, Ca ($CaHPO_4$): 31.500 mg, Fe ($FeSO_4$):18.000 mg, Cu (CuSO): 9.000mg, Mn ($MnSO_4$): 1.800 mg, Zn ($ZnSO_4$): 10.000 mg, Co ($CoSO_4$): 108 mg, Se (Na_2SeO_3): 90 mg.

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Biochemical analysis

Biochemical parameters of the feed and ingredients were analyzed using the methods of the Association of Official Analytical Chemists (AOAC, 2016). Briefly, crude protein content was determined via the Kjeldahl method; crude lipid content was assessed by extracting the sample with a hot chloroform solution using a Soxhlet extractor; ash content was quantified by incinerating the sample in a lab furnace at temperatures ranging from 560°C to 600°C until the sample turned white or gray (~ 6h). To determine the dry weight of materials, wet samples and dry samples were dried in an oven at 105°C for 24h and 4-5h to a constant weight, respectively (AOAC, 2016).

Experimental design

Experiment 1

Juvenile whiteleg shrimp (*Litopenaeus vannamei*) weighing 6.0±0.1 g each were sourced from a commercial shrimp nursery. They were acclimatized for one week in a 1 m³ tank containing water at a salinity of 15 ‰ on a diet containing at least 45% protein. Subsequently, groups of 50 shrimps were transferred to 0.5 m³ tanks containing 350 L of water at 15 ‰ salinity. All tanks were aerated with mechanical aeration. The experimental period lasted for 60 days. The three dietary treatments were randomly assigned to tanks and replicated three times; each replication consisted of 50 shrimps cultured in a 0.5 m³ tank. Shrimp were fed pellets of the appropriate diet for their assigned treatment five times each day at 7 am, 10 am, 1 pm, 5 pm, and 9 pm, because dividing the amount of food for shrimp to eat several times/day helps shrimp digest and absorb nutrients from food more effectively. To maintain high water quality in the tanks, 10-20% of the water in each experimental tank was changed each day. Water quality parameters such as dissolved oxygen, pH, and temperature were monitored using a SevenGo Pro meter (Mettler Toledo, USA). Alkalinity and ammonia levels were assessed using a sera KH test (J. Ravnak GmbH & Co., Heinsberg, Germany), while salinity was measured using a refractometer (Atago, JP, Tokyo, Japan). Monitoring checks were performed daily at 7 am and 2 pm. Optimal water conditions for shrimp growth were maintained (temperature: 28.5-29.4°C; dissolved oxygen: 4.6-5.4 ppm; pH: 8.1-8.2; alkalinity: 119.6-121.7 mg/L $CaCO_3$; total ammonia nitrogen (TAN): 0.56-0.63 mg/L; salinity: 15-18‰).

Experiment 2

Shrimp farmers in Vietnam normally supplement pigments to improve the body color of cultured shrimps for a few weeks before harvest. Consequently, a subsequent 21-day trial was conducted to assess the short-term effects of LLP and SP diets on the body color of whiteleg shrimp. Shrimp weighing 0.45 g were cultured in 0.5 m³ tanks containing 370 L of water at 15 ‰, each tank housed 90 shrimps. Shrimps were fed commercial pellets with 43% protein content (CP, Vietnam) for two months. After this time, the diets were shifted to one of three different treatments: 1) shrimps were continued to be fed a diet of feed pellets; 2) shrimps were fed with feed pellets mixed with 10% Spirulina powder; 3) shrimps were fed with feed pellets mixed with 20% LLP. Shrimps were maintained

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on these diets for a further 21 days. The feed was processed by finely grinding the pellets, mixing them with the respective additives (SP or LLP), adding water, and then pressing them into feed pellets. The care and maintenance protocols were as described for Experiment 1. The color and odor of the raw and cooked shrimps were assessed after 21 days of feeding treatment.

Data collection

Data related to shrimp growth were collected before and after the feeding trial (Experiment 1). The number of shrimps in each tank, initial mean weight (Wi), and final mean weight (Wf) of individual shrimp were recorded before (initial shrimp) and after (final shrimp) the experiment. At the end of Experiment 1, parameters of survival, growth performance, and biochemical body composition were determined. The calculated metrics included survival rate (SR) (%), daily weight gain (DWG) (g day^{-1}), specific growth rate (SGR) (% day^{-1}), feed intake (FI) (% shrimp^{-1} day^{-1}), feed conversion ratio (FCR), protein efficiency ratio (PER), and protein retention (PR) calculated as per the formula (v) below (where *t* = time in days) (Lan *et al.*, 2020):

$$\text{SR (\%)} = \frac{Number\ of\ shrimp\ at\ the\ end\ of\ experiment}{Number\ of\ initial\ shrimp} * 100$$

$$\text{DWG (g day}^{-1}\text{)} = \frac{W_f - W_i}{t}$$

$$\text{SGR (\% day}^{-1}\text{)} = 100 * \frac{Ln(W_f) - Ln(W_i)}{t}$$

$$\text{FI (\% shrimp}^{-1}\text{ day}^{-1}\text{)} = 100 * \Sigma\ \text{Food} / (\sqrt{w_i * w_f}\ ^{*}\ \text{t})$$

$$FCR = \frac{\text{Amount of consumed feed in dry matter}}{\text{Weight gain}}$$

$$\text{PER} = \frac{W_f - W_i}{\text{Protein intake}}$$

$$PR = \frac{\text{Protein of final shrimp} - \text{Protein of initial shrimp}}{\text{Protein intake}}$$

Energy content: The caloric content (kJ g^{-1}) was calculated using the formula: [Protein (%) * 23.7] + [Lipid (%) * 39.5] + [NFE (%) * 17.2]. Where, NFE represents non-fiber carbohydrates.

Final shrimp (10 shrimp tank^{-1}) were collected at the end of experiment 1, euthanized by placing them in ice for 5 min. Euthanized shrimp were homogenized before analysis of their chemical composition (such as moisture, ash, protein, and lipid) following the methods of AOAC (2016).

Sensory color and odor method

At the end of Experiment 1 and Experiment 2, three shrimp from each tank were used to assess color and odor by a sensory method following the protocols of Meilgaard *et al.* (1999). The sensory method was based on the standard scale, with an established sensory council consisting of seven people scoring the shrimp for each parameter using a scale from 1 to 9, with an increasing number representing an increase in quality (Meilgaard *et al.,* 1999; Le *et al.*, 2018). The parameters assessed in this way were

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the color and odor of raw and cooked shrimps (cooked shrimps were prepared by steaming for 10 minutes). In terms of raw shrimp, shrimp color is scored as follows: score of 1 to 6: in the range of light to dark, score of 7: glossy and slight grayish (control shrimp color), score of 8-9: very glossy-greenish and beautiful; fresh shrimp odor is scored as follows: score of 1-6: in the range of very fishy smell to strange smell, score of 7: smell of shirmp in the control, score of 8-9: typical and slight fishy smell. Moving to the cooked shrimp, shrimp color is scores as follows: score of 1-6: light orange; score of 7: orange-red (control shrimp color), score 8-9: beautiful and bright red color; cooked shrimp odor is scored as follows: 1-6: in the range of no aroma to strange odor; score of 7: typical aroma (control shrimp color), score of 8-9: narural and very typical aroma (Le *et al*., 2018). Images of the shrimps were captured using a Canon EOS M200 digital camera.

Data analysis

Data collected from the experiments were processed using Excel (Microsoft, Washington, USA). Initial analysis checked for normal distribution (Shapiro-Wilk test) and homogeneity of variance (Levene's test). Differences between dietary treatments were analyzed using one-way ANOVA. The Duncan's Multiple Range *post-hoc* test was used to determine the significance of differences among three treatments. A significance level of $p<0.05$ was set for all statistical tests. Statistical analyses were conducted with SPSS *vs*. 16.0 (IBM Inc., USA).

Results

Growth and survival rate of shrimp

There were large differences in both the growth and survival rates of shrimps among treatments in the 60-day trial. The shrimps in the FM and 20% LLP diet treatments exhibited the lowest and highest values for final weight, DWG, SGR, and SR, respectively. Shrimp in the 10% SP diet treatment also showed enhanced growth relative to the treatment of FM only, but were outperformed by the 20% LLP diet treatment (Table 2).

Table 2: Growth and survival (mean ± SD) rate of shrimp on three different diet treatments after a 60-day feeding trial. Within a column, treatments with the same letters are not significantly different.

Treatment	W_i (g)	W_f (g)	DWG (g day^{-1})	SGR (% g^{-1} day^{-1})	SR (%)
FM	6.10±0.01^{a}	22.7±0.59^{a}	0.27±0.01^{a}	2.19±0.05^{a}	61.3±1.15^{a}
10% SP	6.09±0.12^{a}	24.7±0.50^{b}	0.31±0.01^{ab}	2.33±0.08^{ab}	64.7±1.15^{b}
20% LLP	6.00±0.03^{a}	25.6±1.33^{b}	0.33±0.02^{b}	2.42±0.09^{b}	66.7±1.15^{b}

Wi: initial mean weight, Wf: final mean weight, DWG: daily weight gain, SGR: specific growth rate, SR: survival rate.

Noticeably, the mean final weight and SR of shrimp in the 20% LLP treatment increased significantly by 12.78% and 8.81% in comparison to the FM diet treatment. For the 10% SP diet treatment, the increases in final weight and SR of shrimps achieved compared to the FM diet treatment were 8.81% and 5.55% respectively, a lower performance in comparison to the 20% LLP diet treatment.

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The results of a one-way ANOVA analysis confirmed that there was significantly higher growth performance for shrimps in 20% LLP diet compared to the control (p<0.05), but no significant difference was found between the 10% SP diet and the FM diet (p>0.05).

Feed intake and feed efficiency

The findings of this present study indicated that there were large differences in FI and feed efficiency parameters, such as FCR, PER, and PR across three different dietary treatments (Table 3). Noticeably, the FM diet treatment exhibited the highest feed intake, significantly higher than both the 20% LLP and 10% SP diet treatments (p<0.05). This suggests that although shrimp fed with the FM-based diet consumed more feed overall (which could imply higher palatability with the conventional diet compared to the diets supplemented with LLP and SP), there was lower feed conversion efficiency for this diet.

Table 3: Feed efficiency of shrimp on three different diet treatments after a 60-day feeding trial (mean ± SD). Within a column, treatments with the same letters are not significantly different.

Treatment	FI (% shrimp^{-1} day^{-1})	FCR	PER	PR
FM	3.34±0.11^{c}	1.59±0.06^{c}	1.65±0.07^{a}	33,3±0.03^{a}
10% SP	3.15±0.06^{b}	1.44±0.06^{b}	1.89±0.06^{b}	36.8±0.33^{b}
20% LLP	2.98±0.05^{a}	1.33±0.01^{a}	2.07±0.06^{c}	39.7±0.17^{c}

FI: feed intake, FCR: feed conversion ratio, PER: protein efficiency ratio, PR: protein retention.

Further analyses of the feed efficiency metrics demonstrated that the 20% LLP diet treatment showed the highest performance in the measured parameters of FCR, PER, and PR with statistically significant improvements over both FM only and 10% SP treatments (p<0.05). The results suggested that treatment of 20% LLP not only enhanced the effective utilization but also improved the overall metabolic efficiency of shrimp. Such improvements in feed efficiency are crucial for sustainable aquaculture practices, reducing waste and enhancing the growth potential of shrimp. In addition, the 10% SP diet also showed significantly better FCR and PER compared to FM only diet (p<0.05), indicating that Spirulina algae also contributed positively to feed efficiency.

Biochemical composition

The biochemical compositions of shrimp, including moisture, ash, lipid, and protein were similar across different treatments (Table 4). Consequently, statistical analysis by one-way ANOVA found that there were no significant differences in the biochemical compositions of shrimps among the three treatments (p>0.05), with the exception of lipid content in the 20% LLP treatment. Lipid levels varied from 1.74% to 2.33% with the highest lipid content observed in the treatment of FM only (2.33%) and the lowest value recorded in the 20% LLP treatment (1.74%). Protein content in shrimp meat was consistent across treatments, ranging from 18.62% to 18.74%. Similarly, the energy composition of shrimp did not vary appreciably between treatments ranging from 5.83 to 6.09 kJ g^{-1}.

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Table 4: Biochemical compositions of shrimp on three different diet treatments after a 60-day feeding trial (mean ± SD). Within a column, treatments with the same letters are not significantly different.

Treatment	Moisture (%)	Ash (%)	Lipid (%)	Protein (%)	Energy (kJ g^{-1})
FM	73.1±1.03[a]	2.31±0.01[a]	2.33±0.11[b]	18.62±0.62[a]	5.83±0.15[a]
10% SP	73.9±0.10[a]	2.50±0.04[a]	2.32±0.05[b]	18.63±0.36[a]	5.90±0.03[a]
20% LLP	73.1±0.53[a]	2.54±0.12[a]	1.74±0.32[a]	18.74±0.59[a]	6.09±0.13[a]

Color and odor of raw and cooked shrimp

The impact of dietary supplementation on the coloration of shrimp is one of the crucial aspects of aquaculture because reddish shrimp color is an indicator of shrimp quality and acceptability for consumers. The results from this present study demonstrated a clear relationship between the color of the diets and the resultant coloration of the shrimp. Treatments of 10% SP and 20% LLP resulted in an improvement in the assessed color and odor of both raw and cooked shrimps in comparison with FM only treatment in both feeding experiments. Consequently, significant differences in the color and odor of raw and cooked shrimps from the 10% SP and 20% LLP diet treatments compared to that of FM diet treatment were found. Although raw shrimps from the 10% SP treatment in both experiments had improved color compared to 20% LLP treatment, cooked shrimps from 20% LLP treatment showed a slightly more reddish color than that of 10% SP treatment (Tables 5 and 6, Fig. 1).

Table 5: Perceived color and odor of shrimps on three different diet treatments after a 60-day feeding trial (mean±SD). Within a row, treatments with the same letters are not significantly different.

Treatment	FM	10% SP	20% LLP
Raw shrimp			
Color	7.57±0.06[a]	8.17±0.15[b]	8.14±0.09[b]
Odor	8.20±0.20[a]	8.87±0.06[b]	8.81±0.01[b]
Cooked shrimp			
Color	7.43±0.15[a]	8.89±0.15[b]	8.94±0.08[b]
Odor	7.70±0.10[a]	8.13±0.15[b]	8.61±0.15[c]

Table 6: Color and odor of shrimps from three different diet treatments after a 21-day feeding trial (mean±SD). Within a row, treatments with the same letters are not significantly different.

Treatment	FM	10% SP	20% LLP
Raw shrimp			
Color	6.60±0.10[a]	8.00±0.10[b]	7.70±010[b]
Odor	8.40±0.20[a]	8.87±0.06[b]	8.67±0.18[b]
Cooked shrimp			
Color	6.46±0.64[a]	8.60±0.26[b]	8.87±0.12[b]
Odor	6.67±0.06[a]	8.00±0.10 [b]	8.00±0.10[b]

Noticeably, the mean score for the color of cooked shrimp in the 20% LLP treatment was 8.94, significantly higher than that of 10% SP treatment (8.87 score) and FM only treatment (only 7.43 score) after the 60-day feeding period (Fig. 1-D). A similar trend was observed in the short-time feeding trial (21 days). In general, scores of color and odor of shrimps assessed by a council in shrimps from the treatments of 10% SP and 20% were scored above 8.00 (except raw shrimp color in 20% LLP after a 21-day

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feeding experiment) and were significantly higher than that in FM treatment in both the 60- and 21- day feeding trials.

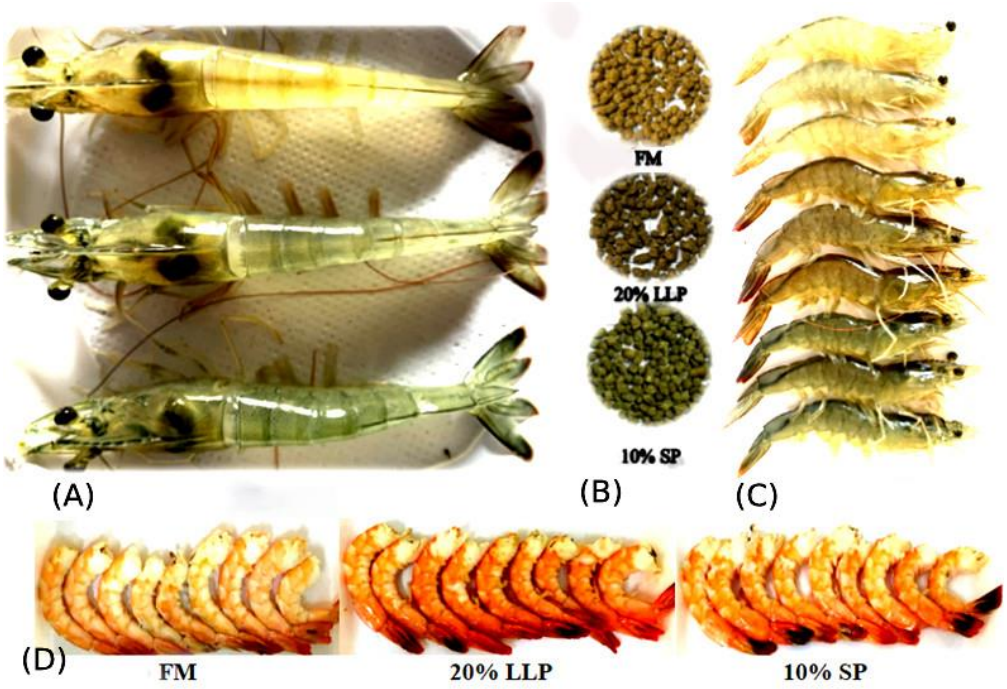


Figure 1: The coloration of raw cooked shrimps from three different diet treatments; (A): color of raw shrimps in a 60-day feeding trial (corresponding with feed color); (B) color of three types of diet; (C) color of the raw shrimps in 21-day feeding trial (corresponding with diet color); (D): color of cooked shrimps in a 60-day feeding trial.

Discussion

Growth and survival rate

Previous studies have shown that *Leucaena* leaf powder (LLP) when used as a replacement for expensive commercial feed in aquaculture such as FM and soybean meal, can have positive influences on the growth and survival rate of aquatic animals, including whiteleg shrimp (Mamat *et al.*, 2017; Chalaune *et al.*, 2022; Lan and To, 2023). In this present study, the mean final weigh (Wf), daily weight gain (DWG), specific growth rate (SGR), and survival rate (SR) of shrimp in FM diet treatment were significantly lower (22.7 g, 0.27 g day^{-1}, 2.19% g^{-1} day^{-1}, and 61.3%, respectively) than that in the diets with 10% SP and 20% LLP. Furthermore, the Wf and SR of shrimp in the treatment with a diet containing LLP 20% were the highest, but did not significantly differ from the 10% SP treatment (Table 2). These results are in agreement with those found by the study of Lan and To (2023) using up to 20% LLP to replace FM for whiteleg shrimp. The study's results showed that the parameters of Wf, DWG, and SR achieved a slightly higher performance in comparison with the control, which only used FM (Lan and To, 2023). Similarly, Chalaune *et al*. (2022) determined that the use of 10% and 20% replacements of Soybean meal by LLP improved Wf and SCR of Nile Tilapia fish after a three-month feeding period.

In addition, numerous studies have shown positive effects of supplementing the diet

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with Spirulina on the growth parameters of fish and shrimp (Kohal *et al.*, 2018; Zidan *et al.*, 2021; Novriadi *et al.*, 2022; Li *et al.*, 2022). For example, Novriadi *et al.* (2022) showed that supplements of 0.2-0.8% of Spirulina meal increased biomass, final body weight, and weight gain of whiteleg shrimp (*L. vanamei*) after 60 days. Kohal *et al.* (2018) demonstrated that partial replacement of FM by Spirulina meal within the range of 1, 3, 5, 8, and 10% improved final weight, specific growth rate, and survival rate of red cherry shrimp (*Neocaridina davidi*) compared with the control treatment. In general, the findings of this study highlighted the efficacy of 20% LLP as a feed supplement in sustainable aquaculture, enabling lower FM content and higher plant-based compositions. This may be partly attributed to a medium-high protein content and other nutrients such as minerals, amino acids, and vitamins in LLP (De Angelis *et al.*, 2021).

Feed intake and feed efficiency

The current study revealed intriguing insights into how Spirulina algae (SP) and *Leucaena leucocephala* powder (LLP) affect shrimp behavior and physiological responses, particularly in food amount consumed and growth performance. The results of this study indicated that both SP and LLP supplementation reduced feed attractiveness compared to the supplemented FM only feed. FM typically has a distinct odor that enhances the shrimp's ability to detect and capture prey, an advantage not seen with plant proteins (Moss *et al.*, 2024). Additionally, LLP contains mimosine, a non-protein amino acid, which further diminishes the prey capture efficiency of shrimps (Akande *et al.*, 2010). The lowest feed intake (FI) was observed in the 20% LLP group, showing a significant difference from the other treatments ($p<0.05$). The 10% SP treatment, however, displayed a higher prey capture rate by shrimp than 20% LLP treatment. Jaime-Ceballos *et al.* (2005) noted that SP could enhance feed palatability through bioactive components like nucleotides, amino acids, and carotenoids in the white shrimp *Litopenaeus schmitti*. Although prey capture by shrimp in the LLP and SP treatments was lower, growth and feed efficiency were improved compared to FM treatment (Table 4), indicating that the nutritional properties of *L. leucocephala* and Spirulina are well-suited for digestion and metabolism in shrimp (Vogt *et al.*, 1986; Li *et al*., 2022). Wilson and Poe (1985) suggested that the amino acid and protein profiles of diets should closely match those of the target livestock for optimal material metabolism. However, this study demonstrates that replacing a portion of FM with vegetable proteins supports better feed conversion in the whiteleg shrimp (*L. vannamei*). This enhancement is likely due to additional nutritional components like minerals and vitamins present in LLP, which aid in improving the metabolism of nutrients in FM-based feeds.

Overall, these findings may partly reinforce the potential of LLP as an effective dietary supplement for improving feed efficiency in shrimp aquaculture. The use of these plant-based supplements not only reduces the reliance on traditional FM, but also enhances the sustainability and

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reduces the environmental footprint of shrimp farming operations.

Biochemical composition

Shrimp are one of the most consumed seafoods thanks to their high-quality protein content combined with high nutritional value for humans (Liu *et al.*, 2021). In this present study, there were significant differences in the lipid content of the whiteleg shrimp in different treatments with a range of 1.74 to 2.33% (d.w.). The shrimp in the treatment of 20% LLP had the lowest lipid content (1.74%). This pattern aligns with the findings of Lan and To (2023), who reported that whiteleg shrimp (*L. vannamei*) on a diet of FM replacement by 20% LLP had the lowest lipid content of 1.81% (d.w.) in comparison with the control and treatments of FM replacement by 5-15% LLP. Pires *et al*. (2018) also found low lipid levels in the white shrimp *L. schmitti*, ranging from 0.86% to 1.4% and Shahkar *et al.* (2014) found that the lipid content of whiteleg shrimp (*L. vannamei*) with different dietary proteins fluctuated from 1.25 to 2.11%. Set aside their high nutritional value, shrimp, including whiteleg shrimp, have a high cholesterol content that may pose a risk of cardiovascular disease if consuming large amounts of shrimp (Dayal *et al.*, 2013; Daniswara *et al.*, 2020). This may be partially offset by feeding shrimp a diet with a partial plant-based composition (*i.e.,* 20% LLP), which the present study showed resulted in better performance and the lowest lipid content in comparison with other diets.

The protein content in shrimp in this present study was consistent across different treatments ranging from 18.62% to 18.74%. Similarly, ash content was also consistent, observed between 2.31% and 2.54%. The energy composition of shrimp also did not vary significantly between treatments, ranging from 5.83 to 6.09 KJ/g. A previous study determined that the meat of black tiger shrimp (*Penaeus monodon*) and white shrimp (*P. vannamei*) had protein contents of 17.1 and 18.8%, respectively (Sriket *et al.*, 2007), whilst the protein content of the whiteleg shrimp meat (*L. vannamei*) was reported to be in the range of 17.23 to 17.89% (Lan and To, 2023). Kohal *et al.* (2018) reported different biochemical values for cherry shrimp (*Neocaridina davidi*) in different diets had notably lower lipid content (0.45% - 0.83%), higher protein content (20.8% - 25.34%), and the lowest ash content (1.40% - 1.62%) in comparison to the results of the present study. There is no doubt that biochemical composition can have large variations depending on dietary components, environmental conditions, and genetic factors of the shrimp.

Color and odor of raw and cooked shrimp

The coloration of shrimp plays an important role in increasing its commercial value. It is well-acknowledged that shrimp color is influenced by the composition of the diet; thus, there were numerous studies conducted in order to improve the coloration of cultured shrimp (Cruz-Suárez *et al.*, 1994; Bernal Rodríguez *et al.*, 2017; Fawzy *et al.*, 2022). In this study, raw shrimp exhibited color changes that mirrored the pigment of their respective feeds. Specifically, shrimp fed with FM displayed the lightest color, followed by

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those on a 20% LLP diet, with an improved greenish color observed in shrimp fed a 10% SP (Figs. 1-A, 1-C). This correlation suggests that the dietary components directly impact the visual pigmentation of shrimp. Further observations on cooked shrimp revealed that the treatment of 20% LLP exhibited a slightly reddish color and a significant color improvement compared to treatments of 10% SP and FM, respectively. Efficiency in increasing the quality of shrimp color by treatments of 20% LLP and 10% SP in comparison with FM treatment was confirmed by a short-term feeding experiment of 21 days. Furthermore, the odor of shrimp in the treatments of 20% LLP and 10% SP also showed a significant improvement over the control group (Table 6).

Previous studies are in agreement with the findings of this present study on the impact of feed composition on shrimp coloration. For instance, Yu *et al.* (2003) reported that intensively farmed whiteleg shrimp fed industrial diets had reduced color. Hanel *et al.* (2007) documented that replacement of FM by the microalgae Spirulina in feed significantly enhanced the pigmentation and growth rates in shrimp in comparison with the control (no FM replacement by microalgae). Fawzy *et al.* (2022) showed that dietary supplementation of β-carotene could achieve desired coloration (redness) and improve the growth parameters of whiteleg shrimp. These studies collectively suggest that dietary components rich in pigments can significantly influence the color and potentially other qualitative aspects of shrimp.

To sum up, in the present study, the diet of 20% LLP improved the color, odor, and growth parameters of the whiteleg shrimp due to the high plant-based content of carotenoids, protein, and other nutrients (Sethi and Kulkani, 1995; Lan and To, 2023). In addition, the result of a 21-day feeding trial with 20% LLP showed that shrimp color and odor could improve after only a short feeding period. This is very important for production practices in Vietnam, where shrimp farmers often tend to improve the color of farmed shrimp in the few weeks before harvest by adding supplements.

Conclusion

The plant-based treatment of 20% LLP had positive effects on the growth performance parameters of whiteleg shrimp, such as weight gain, survival rate, feed intake (FI) and the feed efficiency parameters of feed conversion ratio (FCR), protein efficiency ratio (PER), and protein retention (PR) in comparison with 10% Spirulina algae (SP) and control (FM). Noticeably, the mean final weight and SR of shrimp in 20% LLP treatment increased significantly by 12.78% and 8.81% in comparison to FM treatment, respectively. In addition, the color and odor of raw and cooked shrimp in 20% LLP treatment in both trials of 60-day and 21-day feeding periods improved significantly in comparison with the control group and did not differ significantly with 10% SP diet. In general, the results of this present study prove that partly using plant-based materials to supplement feed and reduce FM use in aquaculture could be more effective in producing the desired color in shrimp, increasing the

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sustainability of production, and using primary productivity more efficiently. The combined effects of LLP and SP on the growth and feed efficiency of whiteleg shrimp in the feed diet should be investigated in the future. In addition, further studies on the positive effects of using LLP and SP in feed formulation on the growth and yield of whiteleg shrimp, as well as a comparison of the economic efficiency of using LLP and SP with FM on commercial scales, should also be considered.

Conflict of interest

The authors declare no conflict of interest.

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