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Effects of replacing fish meal with different levels of Lead tree (*Leucaena leucocephala*) leaf powder on growth, survival, digestive enzymes activity, muscle biochemical composition and texture of white-leg shrimp *Penaeus vannamei* Boone, 1931

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ABSTRACT

This study investigated the effects of replacing fish meal with *Leucaena leucocephala* leaf powder on survival, growth performance, digestive enzyme activities, biochemical body composition and muscle texture of the white-leg shrimp (*Penaeus vannamei* Boone, 1931). Five different diets were examined, by replacing fish meal with *L. leucocephala* leaf powder at 0, 5, 10, 15 and 20%, in triplicates. Shrimps were stocked in 0.5 m^3 tanks @ 70 shrimps per tank and fed on the experimental diets for a period of 60 days. The results showed that the survival rate (%), daily weight gain (g day^{-1}), specific growth rate (% day^{-1}), feed intake (% $fish^{-1}$ day^{-1}), feed conversion ratio, protein efficiency ratio, and protein retention were not significantly different (p>0,05) among the treatment groups. The values of α-amylase and pepsin in the stomach and chymotrypsin (mU min^{-1} mg $protein^{-1}$) in the intestine were not significantly different among treatments. However, the value of α-amylase in the intestine was significantly higher in animals fed diets that replaced fish meal with *L. leucocephala* leaf powder at 0, 5 and 10% compared to other treatments. The biochemical constituents (except for lipid) and the texture (N) of shrimp meat were not significantly different among treatment groups. The results suggested that *L. leucocephala* leaf powder could replace fish meal up to 20% in white-leg shrimp diets.

Keywords: Digestive enzyme, Growth, *Leucaena leucocephala*, White-leg shrimp

Introduction

Aquaculture production has been increased to meet the demand of the growing population (FAO, 2020). Fish meal is recognised as the most important protein source in the aquaculture industry because of its nutrient values (Ayisi *et al*., 2017) and a large portion of fish meal in aquaculture, estimated at 71%, is made from marine wild catch (Boyd *et al*., 2022). Nevertheless, the wild catch fish meal source is likely to be a challenge to satisfy the demand for aquaculture, leading to an increase in the price of fish meal in the last several decades (Olsen and Hasan, 2012).

Shrimp farming has expanded to at least 50 countries worldwide, although shrimp production is mainly from two major regions, Asia and the Americas (Jory, 2018). Among the farmed shrimp species, white-leg shrimp (*Penaeus vannamei* Boone, 1931) is the main species cultivated globally. It contributes to 82.71% of global farmed shrimp production, which comes mainly from six countries *viz*., China, Indonesia, India, Ecuador, Vietnam and Thailand (Boyd *et al*., 2021a). The significant increase in the production of white-leg shrimp, approximately from 2.6 million t in 2010 to 4.9 million t in 2018, consumed a significant quantity of fish meal for this sector (FAO, 2020). According to Boyd *et al*. (2021b), 0.65 t of wild fish is needed to provide 1 t of white-leg shrimp. Ideally, there is a need to reduce dependency of the aquaculture sector on marine sources. Therefore, alternative materials for fish meal in aquaculture, including for white-leg shrimp feed, have been extensively investigated. Previous studies stated that several plant protein sources such as soybean meal, canola meal and blue-green microalga meal could replace fish meal from 20 to 50% in shrimp feed without negative effects on survival and growth (Suarez *et al*., 2009; Macias-Sancho *et al*., 2014; Yang *et al*., 2015).

Lead tree (*Leucaena leucocephala*), known as the Binh Linh tree in Vietnam, adapts well to a wide range of tropical and subtropical areas, particularly in seasonally dry tropical areas (Hughes, 2010). Lead tree is a shrub that is maximum 20 m tall with a bole of 5-50 cm diameter, 4 to 9 pairs of pinnae per leaf and 13-21 pairs of leaflets per pinnae (Verma, 2016). *L. leucocephala* is used for multiple purposes, including timber, fuelwood, forage, organic fertiliser and livestock feed (Olckers, 2011). According to Shelton and Brewbaker (1994), the leaves of *L. leucocephala* contain a relatively high protein level of approximately 25-27%. The high source of vitamins and carotenoids and the amino acid in leaves are well balanced. *L. leucocephala* can be used as a partial replacement for soybean meal in African catfish diets (Sotolu, 2008), fish meal in freshwater prawn diets (Nawwar *et al*., 2017) and Nile tilapia diets (Wee and Wang, 1987). In Vietnam, *L. leucocephala* is widely distributed and used for feeding in animal husbandry sector. Man *et al*. (1995) reported that *L. leucocephala* is the most palatable leguminous species for goat. However, there is only very little information on the practical application of *L. leucocephala* in shrimp aquaculture.

This study examined how replacing fish meal with *L. leucocephala* leaf powder affects survival, growth, digestive enzymes activity, biochemical body composition and muscle texture of white-leg shrimp. The goal was to find materials that can provide inexpensive plant-based protein to replace fish meal protein in shrimp farming, particularly in white-leg shrimp culture.

Materials and methods

L. leucocephala leaf powder (LLP) preparation

L. leucocephala leaves were collected from the rural area in the Tra Vinh Province in South Vietnam. The leaves were collected from small, shrubby and highly branched trees which were approximately 2.5 m tall and having bole of 5 cm in diameter. The leaves were soaked in water for 24 h, dried at 50°C for 72 h, powdered and stored at 4°C until use.

Diet preparation

Ingredients used in this study for diet preparation included fish meal (made from shredded dried fish collected at Dinh An Fishing Port, Tra Vinh Province, Vietnam), soybean meal (VNP Group, Vietnam), soy protein isolate (VMC Group, Vietnam), vitamin-mineral premix, squid oil and hydrolysed protein (Vemedim, Vietnam), rice bran and refined flour. The LLP replaced fish meal at 0, 5, 10, 15 and 20%. All ingredients used were finely ground, analysed for biochemical composition and used for feed formulation with protein level adjusted to 40% in the diets (Table 1). All ingredients were mixed well with water, pelletised, pellets were dried at 60°C for 24 h and stored in plastic containers until use. Diets were analysed for biochemical composition following AOAC methods (AOAC, 2016). Crude lipid was analysed by petroleum ether extraction using a Soxhlet extractor. The ash content was determined after combustion of samples in a furnace at 560-600°C for 6 h. Crude protein was analysed using the Kjeldahl method. The dry matter and feed were determined by drying the samples at 105°C for 4-5 h to a constant weight.

Experimental design

Post-larvae (PL 12) of specific pathogen free (SPF) *P. vannamei* were nursed in a 1 m^3 composite tank about 1 month before being used for experiment. After the nursery period, shrimps (0.94±0.06 g) were randomly assigned to 0.5 m^3 composite tanks containing 20 ppt seawater with aeration. The stocking density was 70 shrimps per

Table 1. Ingredients and proximate composition of 5 different diets (% dry weight)

Ingredients	Percentage replacement of fish meal by LLP				
	0	5	10	15	20
Fish meal	37.00	35.15	33.30	31.45	29.60
Soybean	11.50	12.00	12.50	13.00	13.50
Soy protein isolate	14.50	15.00	15.50	16.00	16.50
Rice bran	28.50	27.50	26.50	25.50	24.50
LLP	-	1.85	3.70	5.55	7.40
Squid oil	2.00	2.00	2.00	2.00	2.00
Vitamin mineral premix[1]	2.00	2.00	2.00	2.00	2.00
Refined flour	3.00	3.00	3.00	3.00	3.00
Hydrolysed protein	1.50	1.50	1.50	1.50	1.50
Total	100.00	100.00	100.00	100.00	100.00
Proximate composition					
Dry matter	87.67	89.00	88.44	87.67	87.89
Crude protein	40.01	39.90	39.94	39.93	39.86
Crude lipid	5.02	5.27	6.22	6.39	6.29
Ash	16.54	15.48	14.45	15.56	15.57
NFE	38.34	39.35	39.40	38.11	38.28
Energy (KJ g^{-1})	18.08	18.70	18.67	18.54	18.51

[1]Vitamin mineral premix: Vitamin A: 1,000,000 IU, Vitamin D: 2,000,000 IU, Vitamin E: 13,300 mg, Vitamin B1: 1,500 mg,Vitamin B2: 3,000 mg, Vitamin B5: 13,340 mg; Vitamin B6: 2,000 mg, Vitamin K3: 1,000 mg, Vitamin C: 12,000 mg, Folic acid: 1,330 mg, Niacin: 7,000 mg, Mn ($MnSO_4$): 150-450 mg, Zn ($ZnSO_4$): 400-850 mg, Mg ($MgSO_4$): 11,000-14,000 mg

tank. Shrimp were fed diets replaced fish meal with LLP at 0, 5, 10, 15 and 20%, in triplicates. Shrimps were fed 5 times daily (7; 10; 13; 17 and 21 h). The experimental period was 60 d. Environmental parameters, such as salinity, pH, dissolved oxygen, temperature, ammonia and alkalinity, were measured twice daily. Salinity was measured using Refractometer (Atago, JP), pH by pH meter (SevenGo; Mettler Toledom, USA), dissolved oxygen by oxygen meter (SevenGo Pro; Mettler Toledo, USA), temperature by temperature meter (SevenGo; Mettler Toledo, USA), ammonia and alkalinity by sera test kit (Sera, DE). All environmental parameters were maintained at suitable levels for shrimp growth (salinity: 18-20 ppt; pH: 7.94-8.16; dissolved oxygen: 4.62-5.42 mg l^{-1}; temperature: 26.49-28.54°C; ammonia: below 0.07 mg l^{-1}, alkalinity: 120-135 mg l^{-1} $CaCO_3$).

Data collection

At the end of the feeding trial, shrimps were observed for survival, growth performance, digestive enzyme activity, biochemical body composition and muscle texture. Survival and growth performance, including survival rate (SR) (%), daily weight gain (DWG) (g day^{-1}), specific growth rate (SGR) (% day^{-1}), feed intake (FI) (% $fish^{-1}$ day^{-1}), feed conversion ratio (FCR), protein efficiency ratio (PER) and protein retention (PR) were determined following the description of Lan *et al*. (2020) and Sarma *et al*. (2022). At the end of the experiment, 9 shrimps were collected from each tank for digestive enzyme analysis, after starving for 24 h. Shrimps were killed by placing them on ice for 5 min, dissected and their stomachs and intestines were removed for analysis. Samples were minced in a buffer solution (20 mM monopotassium phosphate and 6 mM sodium chloride) at pH 6.9. The supernatant was collected after centrifugation at 4,200 rpm for 30 min and stored at -80°C until analysis. The α-amylase content in the stomach and intestine was analysed following the method of Bernfeld (1995). Pepsin activity in the stomach and chymotrypsin activity in the intestine were analysed following methods described previously (Worthington, 1988). The biochemical compositions of shrimp meat such as moisture, ash, lipid and protein were analysed following the protocol described by AOAC (2016). The texture of fresh meat was determined using TA.Xt plus Texture Analyser (Stable Micro Systems, YL, UK) with P75S probe.

Statistical analyses

Before statistical analyses, data were checked for normal distribution and variance homogeneity. One-way ANOVA was applied to test for the effect of fish meal replacement by *L. leucocephala* leaf powder on survival, growth performance, biochemical body composition and muscle texture of the white-leg shrimp. The Tukey's HSD tests were employed for specific comparisons, wherever appropriate. Differences were considered significant at $p<0.05$. All statistical analyses used SPSS software (version 20.0, IBM, USA).

Results and discussion

Survival and growth performance

The survival and growth performance of shrimp fed five different diets are shown in Table 2. There was no significant difference in the survival rate of animals ($p>0.05$) among treatments. Shrimps among treatments also showed no significant difference in the growth rate, including DWG and SGR, although a higher SGR was detected in shrimps fed diets with 20% of fish meal replaced with LLP compared to other treatments.

After 60-days culture, although animals fed diets replaced with 20% LLP displayed better performance in FI, FCR, PER and PR, there were no significant differences among treatments (Table 3).

The obstacle in using plant-based protein in aquaculture diets is that they may contain anti-nutritional factors that may affect the growth of animals (Emire, 2013). This study demonstrated that white-leg shrimp fed fish meal replacement diets using LLP provided similar survival and growth performance in animals fed a diet without fish meal replacement. Notably, slightly better performance of FI and PER was recorded in animals fed a diet with replacement of fish meal by LLP at 20%. Similarly, the recent research by Nawwar *et al*. (2017) supported that *Macrobrachium rosenbergii* fed diet having fish meal replaced with *L. leucopephala* meal up to 25% had a similar specific growth rate and daily

Table 2. Survival and growth performance of shrimps fed 5 different diets after 60 days of experimental feeding

Treatments	SR (%)	Initial weight (g)	Final weight (g)	DWG (g day^{-1})	SGR (% day^{-1})
0%	86.7±4.60	0.93±0.04	15.50±0.65	0.25±0.01	4.76±0.14
5%	87.6±5.40	0.95±0.00	15.71±1.03	0.25±0.02	4.75±0.12
10%	87.1±4.30	0.92±0.02	15.23±0.42	0.25±0.01	4.76±0.03
15%	88.6±7.10	0.97±0.03	15.52±0.01	0.25±0.01	4.71±0.06
20%	94.3±2.50	0.93±0.06	15.84±0.54	0.25±0.01	4.81±0.17

Data are presented as mean±SD

Table 3. FI, FCR, PER and PR of shrimp fed 5 different diets after 60 days experimental feeding

Treatments	FI	FCR	PER	PR
0%	2.05±0.12	1.54±0.12	1.85±0.15	33.3±2.21
5%	1.97±0.09	1.53±0.07	1.85±0.08	32.8±1.51
10%	2.01±0.04	1.55±0.05	1.83±0.06	32.8±1.01
15%	1.98±0.13	1.56±0.13	1.84±0.16	33.7±0.29
20%	1.87±0.06	1.42±0.05	2.01±0.07	35.0±1.97

Data are presented as mean±SD

growth rate compared to a diet without *L. leucopephala* replacement. Another study by Sotolu and Faturoti (2011) on the effect of replacing fish meal with *L. leucocephala* seed meal (LSM) on the growth performance of catfish *Clarias gariepinus* juveniles also reported that there was no significant difference between the weight gains, PER, FCR and SGR of fish fed on 0 and 25% LSM diets. Other plant proteins are potential sources for fish meal replacement in aquaculture. Amaya *et al*. (2007) reported that for white-leg shrimp, under pond conditions, fish meal can be replaced with a combination of up to 39.6% soybean meal and up to 4.8% corn gluten meal without negative effects on growth, FCR and survival. Another study by Yun *et al*. (2017) indicated white-leg shrimp fed diets replaced fish meal with soybean meal up to 33% did not affect growth. According to Akande *et al*. (2010), the leaves contain mimosine recognised as a free amino acid that is likely not beneficial for mono-gastric animals such as fish and prawns and tannins in the leaves of plants has a bitter taste and it may reduce feed palatability and nutrient absorption. However, results in this study and previous studies showed no negative effect of plant-based protein diets. The processing technique that involved soaking and drying techniques might reduce the anti-nutrients in plant leaf meal. The proportional nutrient content, such as protein content, high carotenoids and amino acid balance from replacement diets, may have advantages for absorption and metabolism in animals (Shelton and Brewbaker, 1994).

Digestive enzyme activities

The digestive enzyme levels in the stomach and gut of shrimps fed 5 different diets after 60 days period is illustrated in Table 4. There was no significant difference in the mean value of α-amylase in the stomachs of animals among treatments. However, in the intestine, the α-amylase achieved higher values in the treatments with fish meal replacement diets at 0, 5 and 10% compared to treatments with the replacement at 15 and 20%. Regarding pepsin in the stomach and chymotrypsin in the intestine, the values of these parameters were not significantly different among treatments ($p>0.05$).

The digestive enzyme activities could be directly related to the digestion and absorption of nutrients and the growth of animals (Xia *et al*., 2018). Several important digestive enzymes in the hepatopancreas of shrimps such as α-amylase, pepsin and trypsin might affect the digestion and absorption of food (Carrillo-Farnes *et al*., 2007; Mazumder *et al*., 2018). In this study, the highest value of α-amylase was in the intestine of animals fed 0, 5 and 10% fish meal replacement diets, although the value of this enzyme in the stomach was similar among treatments. Roy *et al*. (2018) also noted that α-amylase levels were relatively higher in the gut of shrimp than in other organs, indicating the gut might play an important role in the digestive process of carbohydrates. Song *et al*. (2017) also indicated that the source of protein might affect the activity of digestive enzymes in the hepatopancreas of shrimps. Although significantly higher activity of amylase was recorded on diets that replaced fish meal by 0, 5 and 10% LLP, growth performance was not affected by the replacement diets.

Muscle biochemical compositions and texture

Table 5 indicates no significant difference in moisture, ash, protein value, and muscle texture of shrimps among treatments ($p>0.05$); however, lipid value was significantly higher in shrimps fed diet without fish meal replacement compared to other groups ($p<0.05$).

Table 4. The activities of α-amylase, pepsin and chymotrypsin (mU min mg^{-1} $protein^{-1}$) in the stomach and intestine of shrimps fed 5 different diets after 60 days experimental period

Treatments	α-Amylase (Stomach)	α-Amylase (Intestine)	Pepsin (Stomach)	Chymotrypsin (Intestine)
0%	35.75±4.24^a	15.38±3.46^a	0.05±0.02^a	233.0±63.40^a
5%	41.46±3.93^a	15.32±2.39^a	0.04±0.02^a	209.4±40.00^a
10%	37.27±1.35^a	14.10±3.45^a	0.04±0.01^a	207.6±47.60^a
15%	36.36±6.10^a	10.26±0.46^b	0.03±0.01^a	205.7±54.40^a
20%	38.07±1.49^a	9.97±0.21^b	0.04±0.01^a	198.9±34.42^a

Data are presented as mean±SD. Different lowercase letters in the same column indicate statistically significant differences ($p<0.05$)

Table 5. Muscle biochemical composition and texture of shrimps fed 5 different diets after 60 days experimental period

Treatments	Moisture (%)	Ash (%)	Lipid (%)	Protein (%)	Texture (N)
0%	75.7±0.14[a]	2.47±0.14[a]	2.43±0.21[a]	17.89±0.22[a]	38.33±2.52[a]
5%	76.0±1.11[a]	2.58±0.06[a]	2.06±0.35[ab]	17.73±0.57[a]	36.33± .58[a]
10%	75.7±0.29[a]	2.55±0.02[a]	2.04±0.12[ab]	17.96±0.20[a]	36.33±2.52[a]
15%	75.7±0.29[a]	2.54±0.09[a]	2.02±0.25[ab]	17.50±0.24[a]	37.33±1.15[a]
20%	76.0±0.50[a]	2.60±0.08[a]	1.81±0.04[b]	17.33±0.36[a]	36.33±0.58[a]

Data are presented as mean±SD. Different lowercase letters in the same column indicate statistically significant differences ($p<0.05$)

Shrimps provide high-quality protein and valuable nutrients for the human body (Abdullah *et al*., 2009). The protein value of experimental shrimps in this study was similar to those reported by Sriket *et al*. (2007) for *P. vannamei* and *Penaeus monodon* (18.8 and 17.1%, respectively). In this study, the moisture value of shrimp was also similar to those reported in fresh shrimp (75-80%) (Sambhu and Jayaprakash, 1994; Yanar and Celik, 2006). The ash content of white-leg shrimp was observed at 1 to 1.5% in previous studies (Yanar and Celik, 2006; Gokoglu *et al*., 2008), lower than those recorded in this study. The lipid value of *Exopalaemon annandalei* was at 1.78% (Li *et al*., 2011) close to those observed in this study. Simon *et al*. (2012) recorded a lower lipid level (0.86-1.4%) in *L. schimitti*. Other research recorded a lipid value at 1-1.34% in *Penaeus brasiliensis*, *P. monodon* and *P. vannamei* (Moura and Tenuta, 2002; Sriket *et al*., 2007; Li *et al*., 2011; Puga-Lopez *et al*. 2013; Bragagnolo and Rodriguez-Amaya, 2021). It is noted that the chemical composition in marine fish can vary between species and within the same species because of the effects of several factors, including diet, density, growth stage, quality and salinity of the water and seasonal changes (Saldanha *et al*., 2006; Sriket *et al*., 2007). Regarding texture in shrimp muscle, it is reported that the source of protein influences the texture of shrimp muscle (Ezquerra Brauer *et al*., 2003). However, this study observed that the replacement of fish meal up to 20% of LLP had no effect on shrimp muscle texture.

In conclusion, white-leg shrimp fed diets replacing fish meal by LLP at 5, 10, 15 and 20%, showed similar survival, growth performance, digestive enzymes activity (such as pepsin and chymotrypsin) and most of the biochemical characteristics as well as muscle texture were found similar to shrimp fed diet without LLP replacement. Therefore, LLP could be a potential source to replace fish meal in the white-leg shrimp diet up to 20%.

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