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# Partial replacement of fish meal by white leadtree meal in diets for juveniles of Giant River Prawn, *Macrobrachium rosenbergii* (De Man, 1879)

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**Abstract**
The potential use of white leadtree (*Leucaena leucocephala* Lam.) meal to replace fish meal in diets for *Macrobrachium rosenbergii* (De Man, 1879) juveniles was assessed in a feeding trial. A total of 750 juveniles (approximately 2.0 cm/individual) were randomly assigned to fifteen tanks. Five iso-nitrogenous diets were formulated by replacing 12.5% (Diet 2), 25.0% (Diet 3), 37.5% (Diet 4) and 50.0% (Diet 5) of fish meal by *L. leucocephala* meal. A diet without fish meal substitution served as control treatment (Diet 1). The juveniles were fed the experimental diets for 70 days. Results revealed that juveniles fed with Diet 2 exhibited the highest specific growth rate (SGR) (3.62±0.13%) and daily growth rate (DGR) (0.014±0.0017 g $day^{-1}$) followed by Diets 1 and 3. There was no significant difference ($p$>0.05) in SGR values among juveniles fed with Diets 1, 2 and 3. Juveniles fed with Diets 4 (3.04±0.14%, 0.008±0.0007 g $day^{-1}$) and 5 (2.70±0.13%, 0.006±0.0004 g $day^{-1}$) showed lower SGR and DGR values than those fed with the other diets. Results of the present study indicate that *L. leucocephala* meal could replace fish meal up to 25% in diets for *M. rosenbergii* juveniles.

**Keywords:** *Leucaena leucocephala*, fish meal replacement, giant river prawn

## 1. Introduction
In freshwater aquaculture, apart from carp, some of the largest growing prawn species of *Macrobrachium* genus, particularly *Macrobrachium rosenbergii* (De Man, 1879), or commonly called 'Scampi' has been found to be more suitable for rearing. Hatchery-rearing of the giant river prawn (*M. rosenbergii*) started in Malaysia in the early 1960's with the discovery of brackish conditions for the juveniles [1, 2]. *M. rosenbergii* has remarkable advantage, because of its omnivorous feeding habit, high growth rate, attractive size, better meat quality with good amount of protein, tolerance to wide range of temperature (15-35°C) and resistance to diseases [3].
The increasing production of *M. rosenbergii* is associated with higher food demands. Cultured *M. rosenbergii* are generally fed with formulated diets (fish pellets). Fish meal has become the main ingredient in the formulation of pellets for aquaculture uses [4]. However, fish meal supplies have been dwindling due to overfishing, elevating prices, diminishing natural fish stock and market volatility. Therefore, many terrestrial plant materials have been used to substitute fish meal in the feeding of farmed organisms [5- 9].
*Leucaena leucocephala* (Lam.), the white leadtree or also known as 'petai belalang' in Malaysia and 'petai cina' in Indonesia, essentially grows in tropical areas because it requires warm temperature for optimal growth. Normally, all segments of *L. leucocephala* like pods, leaf and seeds are used as a food. In Indonesia, it is also used to make coffee substitute. The leaves of *L. leucocephala* contain approximately 25-27% protein and high β-carotene. The amino acid in leaves are well balanced, contain high source of vitamins and carotenoids [10]. An earlier study has evaluated the use of *L. leucocephala* in African catfish diets. They concluded that *L. leucocephala* could be used to replace fish meal up to 20% without adverse effect on the growth performance of the fish [11].
An understanding about nutrition of cultured organisms is the most valuable knowledge to develop inexpensive diets and increase profitability in aquaculture industries. Therefore, the present study was carried out to compare the growth performance of *M. rosenbergii* juveniles fed with diets formulated from *L. luecocephala* meal at 0%, 12.5%, 25%, 37.5% and 50% inclusion levels.

## 2. Materials and Methods
### 2.1 Experimental setup
Juveniles of *M. rosenbergii* with an average length of 2.0 cm/individual were obtained from a local commercial hatchery. The juveniles were transported to the Aquaculture Laboratory at Universiti Teknologi MARA Perlis, Malaysia. A hundred individuals were bulk-weighed to determine the initial body weight. Fifty juveniles were randomly distributed into each tank with a dimension of 60 cm × 40 cm × 30 cm. During the feeding trial, the juveniles were fed twice per day, at 0800 and 1600 hours. They were fed at 7% of their total body weight. Dissolved oxygen, temperature rate and pH readings were monitored daily to maintain their optimal levels. The feeding trial was carried out for 70 days between April and June 2016.

### 2.2 Preparation of experimental diets
Five iso-nitrogenous diets were prepared in this study. Fish meal was replaced with *L. leucocephala* meal at 0% (Diet 1), 12.5% (Diet 2), 25.0% (Diet 3), 37.5% (Diet 4) and 50.0% (Diet 5). The formulation and proximate composition of the experimental diets are shown in Table 1. Each experimental group was triplicated. The dry ingredients including *L. leucocephala* meal, fish meal, wheat flour and wheat bran were ground to reduce the size and mixed together thoroughly. Then, the choline chloride, binder, vitamin, mineral, fish oil and corn oil were added to the mixture. Water was added to the mixture to form mash. The mixture was then extruded using a household meat mincer. The spaghetti-like strands were cut into 1 mm length. The pellets were dried in an oven at 60 ˚C for 24 hours until constant weight. The pellets were stored in bags to keep out moisture.

### 2.3 Proximate Analysis
Total protein, total lipid, total carbohydrate, ash and moisture were determined for each diet. The proximate analysis was conducted according to standard methods described by Association of Official Analytical Chemists [12].

### 2.4 Growth Performance
Growth (wet weight) was determined biweekly to minimise handling on the juveniles. Thirty juveniles were randomly sampled from each treatment tank. Growth performance was assessed as follows:

Daily growth rate = Wf - Wi/T;

Specific growth rate = 100 (lnWf - lnWi)/T

*where Wf and Wi are the final and initial weight, respectively, and T is the number of days.

Survival rate of the juveniles was assessed as below:

Survival = 100 (Nf/Ni)

*where Nf and Ni are the number of fish at the start and final of experiment.

### 2.5 Statistical Analysis
All data were analysed with parametric statistics using the SPSS version 17.0 statistical software. Data with proportions were transformed by arcsine square root transformation. The comparison among the means was analysed using one-way analysis of variance (ANOVA) followed by Tukey's HSD *post hoc* tests to determine the differences of means between the treatments.

**Table 1:** Formulation (for 100 g) and proximate composition of five iso-nitrogenous diets. Different letters in subscript following values denote statistical difference ($p$<0.05).

| **Ingredient (g)** | **Diet 1** | **Diet 2** | **Diet 3** | **Diet 4** | **Diet 5** |
|---|---|---|---|---|---|
| Fish meal | 50.0 | 53.5 | 46.1 | 36.4 | 30.7 |
| *L. leucocephala* | - | 14.5 | 28.8 | 43.2 | 57.6 |
| Wheat flour | 28.5 | 12.0 | 8.6 | 5.2 | 1.9 |
| Wheat bran | 28.5 | 12.0 | 8.6 | 5.2 | 1.9 |
| Fish oil | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 |
| Corn oil | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 |
| Choline chloride | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Vitamin and mineral | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 |
| Binder | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| **Treatment** | **Total protein (%)** | **Total lipid (%)** | **Ash (%)** | **Moisture (%)** | **Total carbohydrate (%)** |
| Diet 1 | $39.72\pm3.15_a$ | $2.73\pm0.31_a$ | $2.67\pm0.03_a$ | $2.61\pm0.03_a$ | $51.27\pm3.17_a$ |
| Diet 2 | $39.48\pm3.38_a$ | $3.31\pm0.21_a$ | $3.58\pm0.06_b$ | $2.64\pm0.05_a$ | $50.99\pm3.49_a$ |
| Diet 3 | $38.61\pm1.39_a$ | $3.61\pm0.46_a$ | $4.28\pm0.05_c$ | $2.79\pm0.08_{a,b}$ | $50.71\pm1.44_a$ |
| Diet 4 | $38.78\pm1.67_a$ | $5.97\pm0.49_b$ | $5.62\pm0.1_d$ | $3.54\pm0.19_b$ | $46.08\pm2.12_a$ |
| Diet 5 | $39.66\pm0.80_a$ | $9.78\pm1.25_c$ | $6.83\pm0.24_e$ | $6.65\pm0.93_c$ | $36.39\pm2.26_b$ |

## 3. Results and Discussion
### 3.1 Growth performance of *M. rosenbergii* with experimental diets
Figure 1 shows the Specific Growth Rate (SGR) of *M. rosenbergii* fed with the experimental diets. Juveniles fed with Diet 2 exhibited the highest SGR value (3.62±0.13%) while the lowest value was displayed by juveniles fed on Diet 5 (2.66±0.13%). Results also revealed that the growth decreased with the increasing amount of white leadtree meal in *M. rosenbergii* diets. The one-way ANOVA test showed that the SGR values were significantly different among the diets ($p$<0.05). A further Tukey's *post hoc* analysis showed no significant difference in SGR among juveniles fed with Diets 1, 2 and 3. However, SGR values for juveniles fed with Diet 4 and 5 were statistically different from those fed with Diets 1, 2 and 3. The determination of Daily Growth Rate (DGR) of the juveniles displayed similar results to SGR (Figure 2). Juveniles fed Diet 2 exhibited the highest growth increment compared to other treatments. The DGR values of juveniles with Diet 1 (0.014±0.002 g $day^{-1}$) and Diet 3 (0.006±0.0004 g $day^{-1}$) were significantly similar to Diet 2 ($p$>0.05). Poor growth performance was observed in juveniles fed with 50% white leadtree meal (Diet 5). Statistical analysis showed a significant difference in DGR values with juveniles fed on Diets 4 and 5.

*L. leucocephala* has been considered as an excellent source of protein for both ruminant and non-ruminants [13, 14]. Although the leaves of *L. leucocephala* have high nutritive value such as acceptable protein content, elements and minerals, but it reduced the performance of *M. rosenbergii* in the present study. This could be explained by the presence of some anti-nutritional factors that can reduce the improvement of *M. rosenbergii*. The leaves contain mimosine, a free amino acid that is limited for mono-gastric animals like fish and prawn because it affects the thyroid function which might have resulted in poor growth of *M. rosenbergii* when the levels increased. In addition, presence of tannins in the leaves of *L. leucocephala* results in bitter taste and it reduces feed palatability and absorption of B12 [15].

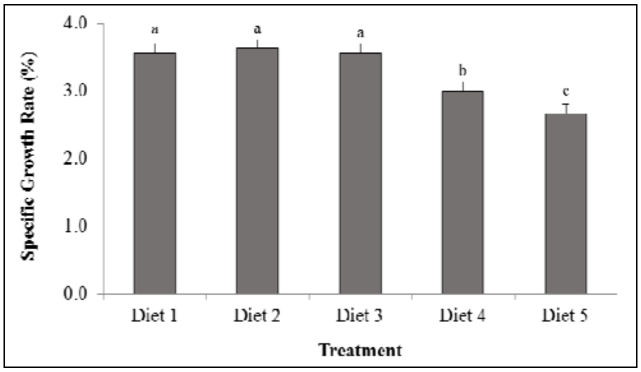


**Fig 1:** The Specific Growth Rate (SGR) of *M. rosenbergii* juveniles fed with different inclusion levels of white leadtree meal. Diet 1: 0%; Diet 2; 12.5%; Diet 3: 25.0%, Diet 4: 27.5% and Diet 5: 50.0%. Different letters on each bar show statistical difference (α=0.05).

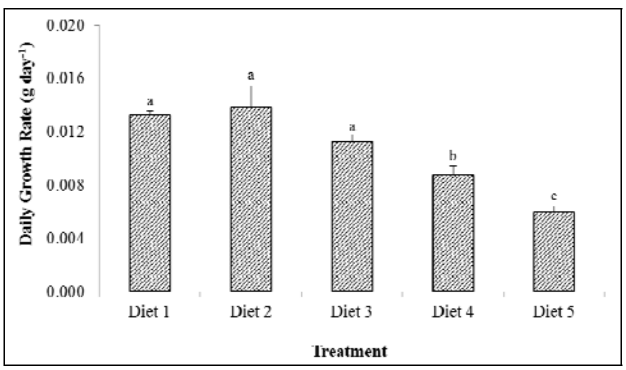


**Fig 2:** The Daily Growth Rate (DGR) of *M. rosenbergii* juveniles fed with different inclusion levels of white leadtree meal. Diet 1: 0%; Diet 2; 12.5%; Diet 3: 25.0%, Diet 4: 27.5% and Diet 5: 50.0%. Different letters on each bar show statistical difference (α=0.05).

An earlier study by Falaye *et al.* showed poor growth performance of *Oreochromis niloticus* when fed on *L. leucocephala* at higher inclusion level due to effect of anti-nutritional factor [16]. The results for reduced growth performance in *M. rosenbergii* are also supported by an earlier study when the shrimp and fish showed the toxicity sign when fed on *L. leucocephala*. The shrimp and fish also showed the reduction in growth rate and feed efficiency [17]. A trend of reduced growth performance and feed efficiency with increasing level of *L. leucocephala* meal also reported in nile tilapia [18].

### 3.2 Survival rate of *M. rosenbergii* juveniles
Figure 3 shows that juveniles on Diet 2 displayed the highest survival rate (80.7±0.75%) while the lowest survival rate was obtained with Diet 4 which is 70.0±0.73%. Results for one-way ANOVA test showed significant difference with different diets ($p$<0.05). A further Tukey's *post hoc* test revealed that survival rate of juveniles fed on Diet 4 was significantly lower from all other treatments ($p$<0.05). Survival rate of *M. rosenbergii* in the treatment diets varied among treatments due to different inclusion levels of *L. leucocephala* meal. Juveniles fed on Diet 4 had the lowest survival rate due to the effect of anti-nutritional factor that might have influenced the mortality. Although there was a significant difference in survival rate between juveniles fed on Diet 4 and Diet 5, the difference was minimum. This finding is in agreement with an earlier study which showed higher mortality in fish with increasing levels of *L. leucocephala* [19].

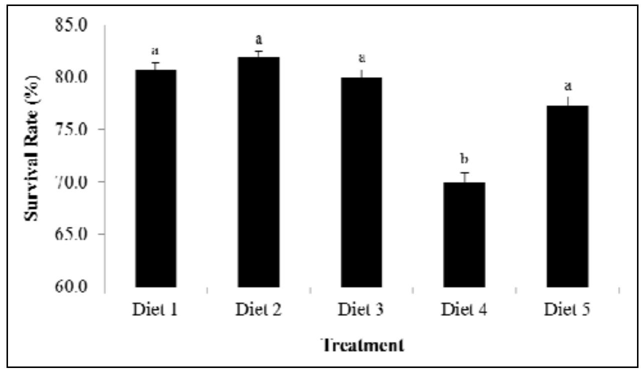


**Fig 3**: The survival rate of *M. rosenbergii* juveniles fed with varying inclusion levels of white leadtree meal over 70 days period. Different letter on the bars show statistical difference (α=0.05).

### 4. Conclusion
The use of *L. leucocephala* meal in diets for *M. rosenbergii* juveniles should be considered as potential regardless of the anti-nutritional effect. Partial inclusion of the plant meal might have significant effect on the growth of the animals and reduction of feeding cost in an aquaculture farm. The outcomes of this study are useful in better understanding of the production of new alternative solution for fish meal replacement.

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