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Effects of Legume-Enriched *Moina micrura* on the Growth, Survival, and Nutritional Composition of Giant Freshwater Prawn (*Macrobrachium rosenbergii*) Post-larvae

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ABSTRACT

Moina micrura, a type of freshwater zooplankton known as water fleas commonly employed as live feed for fish in aquaculture and must be nutritionally enriched to provide the best nutrients for crustacean larvae's development and survival. This study aims to improve the growth, reproduction, and nutritional value of *M. micrura* and to analyse the effects of utilising different types of enriched *M. micrura* on protein, lipid, and growth of giant freshwater prawn *Macrobrachium rosenbergii*. The effectiveness of enriched *M. micrura* on *M. rosenbergii* post-larvae was evaluated using three types of legume-based enrichment (soybean, mung bean, and sesame seed) and yeast as a control for triplicates of each. The growth, survival, protein, and lipid compositions of *M. rosenbergii* post-larvae were determined after 30 days to compare the efficacy of each treatment. The results showed that *M. micrura* fed with soybean had the highest survival rate and specific population

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growth rate, with values of 95.00 ± 4.9% and 0.059 ± 0.001%, respectively. A higher specific growth rate of *M. rosenbergii* was observed when fed with *M. micrura* enriched with soybean (5.73 ± 0.21%), and the lowest was when fed with *M. micrura* enriched with mung bean (3.84 ± 0.23%). Soybean enrichment proved to be the most effective among the different enrichments utilised, particularly in the assessment of the proximate analysis of *M. rosenbergii* post-larvae. The mean values for protein and lipid content in *M. rosenbergii* were determined to be 69.20 ± 0.77% and 7.52 ± 0.55%, respectively. The results indicated that it is highly recommended to use *M. micrura* enriched with soybean to feed *M. rosenbergii* post-larvae.

Keywords: Enrichment, growth, legumes, *Moina micrura*, *Macrobrachium rosenbergii*, nutrients

INTRODUCTION

Moina sp., a type of freshwater zooplankton commonly known as water fleas, is a small species of cladoceran crustacean (Joshue et al., 2022; Othman et al., 2024). A diverse array of zooplankton species has been commonly employed as live feed for fish in aquaculture, with Moina being notable due to their diminutive size and erratic motions, which appeal to larval fish (Lomartire et al., 2021). *Moina* sp. possess significant potential as a live food source for larval fish and crustaceans (Islam et al., 2017). *Moina micrura* is particularly suitable as an early feed due to its small size, quick growth, high rate of reproduction, ease of culture, high content of digestive enzymes, and simplicity in managing their nutritional value. Nevertheless, *M. micrura* must be supplemented with highly nutritious feed prior to being provided to fish fry and shrimp larvae because of their nutritional variability. Singh et al. (2019) reported that *Moina* sp. lacks essential nutrients necessary for the growth and survival of predatory fish and crustacean larvae, particularly in n-3 highly unsaturated fatty acids, such as EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid). Therefore, it is necessary to improve the nutritional quality by enriching zooplankton, including *Moina* sp, through pre-feeding (Das et al., 2007).

The post-larvae (PL) of giant freshwater prawn *Macrobrachium rosenbergii* depend significantly on dietary protein and lipids for growth; however, Moina alone may be insufficient in supplying these nutrients adequately. Protein is a crucial macronutrient essential for tissue development and moulting; its deficiency may result in delayed development and increased mortality rates. Insufficient protein also can result in inadequate muscle development and prolonged moulting cycles, heightening vulnerability to predation and cannibalism. Lipids are essential for energy provision and metabolic functions. Despite the presence of lipids in Moina, the total lipid content may be insufficient to satisfy the energy requirements of *M. rosenbergii* post-larvae, especially in intensive culture systems with high energy demands. Insufficient lipid intake may cause post-larvae to allocate protein for energy metabolism rather than growth, leading to diminished feed efficiency and slower weight gain (Chuphal et al., 2021).

Alternative plant-based feed sources, including soybean (*Glycine max*), mung bean (*Vigna radiata*), and sesame seed (*Sesamum indicum*), present promising nutritional profiles that may improve zooplankton culture. Soybean is known for its high protein content, comprising roughly 35-40% of its dry weight (Modgil et al., 2020). This protein is abundant in essential amino acids, including arginine and glutamine, which are essential for zooplankton development and enzymatic activities. Mung bean provides a moderate protein content of around 20-25%, featuring a balanced amino acid profile that facilitates zooplankton growth and reproduction. Sesame seed is distinguished by its high lipid content (~50%), featuring substantial amounts of polyunsaturated fatty acids (PUFAs) like linoleic acid, which are crucial for zooplankton reproductive success and larval development (Thiruvasagam et al., 2024). Sesame seed comprises approximately 18-25% protein, though its amino acid profile exhibits reduced lysine levels in comparison to soybean and mung bean.

This study aims to enhance the growth, reproduction, and nutritional quality of *M. micrura* by enriching it with three legume-based diets (soybean, mung bean, sesame seed) alongside yeast as a control, and to evaluate the nutritional transfer effects of enriched *M. micrura* on the protein content, lipid profile, and growth performance of *M. rosenbergii* post-larvae.

MATERIALS AND METHOD

Experimental Setup

M. micrura was sampled from Tasik Kenyir, Kuala Berang, Terengganu, Malaysia. Upon collection, the live samples were rinsed with dechlorinated freshwater (1µm) to prevent contamination and were transported directly to the laboratory. Cladocerans were isolated and classified based on their morphological characteristics. Different species of Moina were identified by studying their body shape and segments of the antenna (Bledzki & Rybak, 2016; Patil et al., 2007; Shiel, 1995; Yong & Yule, 2004).

The study was conducted at the Hatchery of the Faculty of Fisheries and Food Science (FPSM), located at Universiti Malaysia Terengganu, for 30 days. The feeding regimen for *M. micrura* involved a once-daily administration, with a concentration of 500mgL^{-1}, as detailed by Suhaimi et al. (2022a) and Naman et al. (2021). The experimental design consisted of four different dietary treatments with soybean (*Glycine max*), mung bean (*Vigna radiata*), sesame seed (*Sesamum indicum*) and yeast as a control. Each condition was tested in triplicate (4 tanks × 3 replicates).

To assess the growth of population density, *M. micrura* was reared in 25L aquarium tanks, with an initial stocking density of 40 individuals per mL. The feed was prepared fresh daily by weighing powdered feeds, dissolving them in dechlorinated freshwater, and filtering the solution through a 50µm mesh. A feed concentration of 500 mgL^{-1} was then introduced into the culture tanks (Khoo et al., 2013; Suhaimi et al., 2022b). Water quality was maintained by replacing 50% of the water every two days, with a temperature of 25-27 °C and a pH of 7.5 (Islam et al., 2017; Suhaimi et al., 2022b).

For this study, post-larvae of *M. rosenbergii* PL-5 were used. Upon their arrival, a systematic acclimatisation process was implemented for the post-larvae prior to their stocking into individual aquariums. The experimental design incorporated three replicates for each treatment, including the control, resulting in a comprehensive set of 12 replicates. The cultivation of *M. rosenbergii* post-larvae comprised a total of 12 replicates, for three treatments and one control, where each tank consisted of a total of 100 post-larvae.

The post-larvae received twice-daily feedings of enriched *M. micrura.* Before each feeding session, the enriched *M. micrura* was extracted from the treatment aquarium, washed, sieved and weighed. To enhance nutritional value, *M. micrura* underwent a 6-hour enrichment process within the treatment tank before each post-larvae feeding, following the methodology outlined by Fereidouni et al. (2013). Aeration systems were installed in all aquariums to ensure optimal cultured conditions. Water exchange was done for 50-70% every week, and water temperature (26-27 °C), pH (3-5), salinity (0 ppt), and dissolved oxygen were maintained throughout the experiment.

Diet Preparation and Enrichment of *Moina micrura*

In this study, three types of legume-based treatments and one control treatment were utilised. The three types of legumes (soybean, sesame seed, and mung bean) were washed with clean water to eliminate any foreign particles. Following the thorough cleaning, the legumes underwent a drying process inside the oven at a temperature of 50 °C to eliminate any remaining moisture while preserving their original nutritional properties. Once the legumes were completely dry, they were ground using a grinder to form a fine powdery texture. These powdered legumes were then used to enrich *M. micrura* by mixing them with dechlorinated freshwater until fully dissolved. The feed was sieved through a 50µm mesh (Khoo et al., 2013) prior to being introduced into the culture tank with 4000 individuals per litre of Moina (Rasdi et al., 2021). *M. micrura* was fed the diets once daily at a concentration of 500mgL^{-1}.

Protein and Lipid Analysis

The analysis of protein and lipid was conducted on diet treatments, enriched *M. micrura* and *M. rosenbergii* post-larvae at the Laboratory of General Analysis and Quality at the Faculty of Fisheries and Food Science, Universiti Malaysia Terengganu. *M. micrura* was enriched sequentially for about 6 hours to enhance the nutritional quality of zooplankton while preventing significant degradation or nutrient depletion (Yuslan et al., 2021). After 6 hours of enrichment every day, the *M. micrura* was harvested and dried.

All prepared samples were pulverised using a mortar and pestle into a powder form (< 0.1 mm) (Suhaimi et al., 2022b). The analysis of crude protein and crude lipid was performed. The protein analysis involved placing approximately 0.2g of samples into glass tubes for the purpose of digestion. Three different methods—digestion, distillation, and titration—were used to determine the protein content. The percentage of protein was calculated by multiplying the N2 (%) percentage by an empirical value of 6.25. The total lipid content of the diets was assessed utilising the modified Bligh and Dyer (1959) method. Approximately 2g of each sample was weighed and underwent lipid extraction. The extraction solvent was evaporated, and the residual lipid fraction was collected in a pre-weighed cup. The cup, containing the extracted lipids, was desiccated to eliminate any remaining solvent prior to reweighing. The total lipid content was determined by the weight difference before and after extraction.

Growth, Survival and Reproduction of *Moina micrura*

The population growth of *M. micrura* was determined by counting the population of *M. micrura* once every two days for 15 days, which was equivalent to the average lifespan (cycle) of *M. micrura.* Aeration was turned off for 5-10 minutes prior to sampling to enable uniform distribution of the *M. micrura* within the culture medium. Sampling was conducted at several points within the culture container to address spatial variability, with each sample comprising a predetermined volume of 10mL collected from a minimum of four points. The *M. micrura* samples were observed in a counting chamber under the microscope.

The specific population growth rate (r) and survival rate were calculated by using Equation 1 and Equation 2 (Lee et al., 2013; Yuslan et al., 2021):

$$\text{Specific population growth rate (r)} = \left(\frac{\ln \mathrm{Ne} - \ln \mathrm{Ni}}{\mathrm{t}}\right) \qquad [1]$$

$$\text{Survival rate} = \left[\frac{\text{Total number of the sample taken}}{\text{Total number of initial } \mathit{Moina} \text{ sp.density}}\right] \times 100\% \qquad [2]$$

where Ni: initial density of *M. micrura*, Ne: end density of *M. micrura,* and t: cultured days.

The reproduction of *M. micrura* was determined with 5 individuals, and the experiment was conducted using the equation by Khoo et al. (2013) to measure reproduction, and the life tables were used to record reproduction data, which included gross reproduction rate, net reproduction rate, and generation time. These data were estimated as follows in Equations 1 to 5:

$$\text{Gross reproduction rate} = \sum \mathrm{m_x} \qquad [3]$$

$$\text{Net reproduction rate } (R_0) = \sum l_x\ m_x \quad [4]$$

$$\text{Generation time (T)} = \sum \frac{l_x\ m_x x}{R_0} \quad [5]$$

where, m_x: age-specific fecundity (number of neonates produced per surviving female at age *x*), and l_x: Proportion individual surviving to age *x*.

Growth Performance and Survival of *Macrobrachium rosenbergii* Post-larvae

At the start of the experiment, the initial body weight (BW) and initial body length (BL) of the post-larvae were measured and recorded; the same were done after 30 days of the experiment, where the final weight and final length of the post-larvae in each tank were measured and recorded in order to determine the survival rate and specific growth rate (SGR) of *M. rosenbergii* post-larvae of each treatment. After the 30-day experimental period, the survival rate and specific growth rate (SGR) of *M. rosenbergii* were individually determined using the subsequent Equations 6 to 7 (Asaduzzaman et al., 2016; Sumon et al., 2018):

$$\%\ \text{Survival rate} = \frac{\text{No.of live PL}}{\text{No.of PL introduces}} \times 100 \quad [6]$$

$$\text{Specific growth rate (SGR)} = (\ln W_t - \text{In } W_i \times 100) / t \quad [7]$$

where W_i: initial weight of *M. rosenbergii* post-larvae, W_t: final weight of *M. rosenbergii* post-larvae, and t: cultured days.

Statistical Analysis

Before conducting the statistical analysis, all recorded data were subjected to a normality test. The Statistical Package for the Social Sciences (SPSS) software was utilised to execute a one-way analysis of variance (ANOVA) on the experimental data, and significant differences were determined employing the Tukey test ($P < 0.05$). Presentation of all data was done in the form of mean ± standard deviation.

RESULTS

Protein and Lipid Content of Diets and Enriched *Moina micrura*

Based on our results in Figure 1, yeast recorded the highest protein (51.41 ± 2.52%) compared to soybean (39.47 ± 1.27%), mung bean (21.08 ± 0.68%), and sesame seed, which was recorded as the lowest (17.16 ± 0.75%). For lipid content, sesame seed recorded the highest (26.61 ± 0.56%) compared to soybean (9.77 ± 0.77%), mung bean (1.02 ± 0.61%), and the least lipid content was recorded in yeast (0.93 ± 0.58%), as stated in Figure 2.

The highest protein content was *M. micrura* enriched with soybean (73.92 ± 0.13%), compared to *M. micrura* enriched with yeast (72.11 ± 0.30%), sesame seed (71.48 ± 0.24%), and the lowest was recorded in mung bean (69.02 ± 0.28%), as stated in Figure 3. The highest lipid content was recorded in *M. micrura* enriched with soybean (14.68 ± 0.65%), followed by *M. micrura* enriched with sesame seed (14.18 ± 0.31%), *M. micrura* enriched with yeast (12.81 ± 0.74%), and the lowest was recorded in *M. micrura* enriched with sesame seed, as stated in Figure 4.

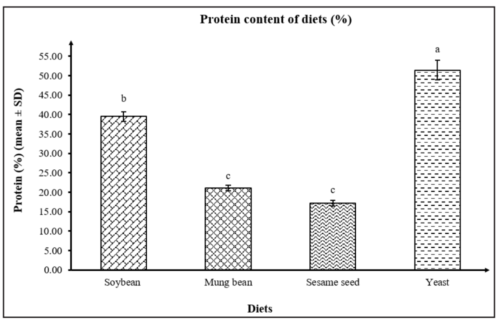

Figure 1. Protein content of diets. The small letters indicate significant differences between enrichments (*P*<0.05)

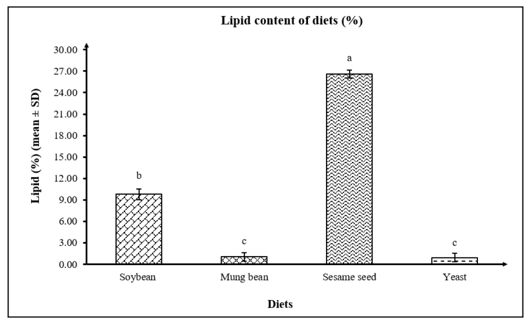

Figure 2. Lipid content of diets. The small letters indicate significant differences between enrichments (*P*<0.05)

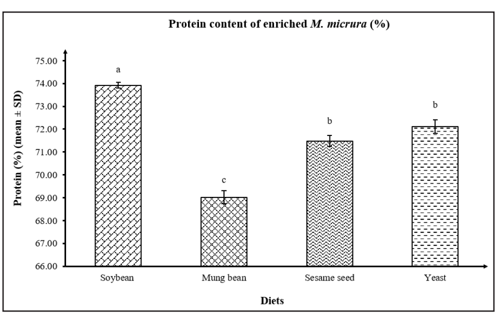

Figure 3. Protein content of enriched *M. micrura* fed with different diets. The small letters indicate significant differences between enrichments (*P*<0.05)

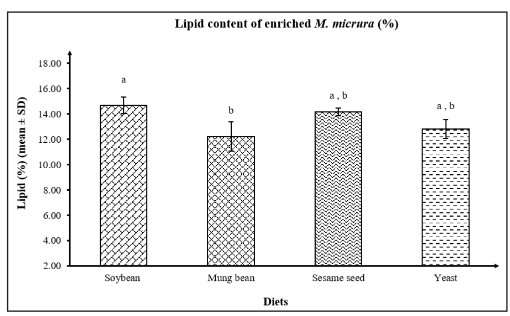

Figure 4. Lipid content of enriched *M. micrura* fed with different diets. The small letters indicate significant differences between enrichments (*P*<0.05)

Growth, Survival, and Reproduction of *M. micrura*

The results showed that *M. micrura* fed soybean had the highest population growth compared to the other diets, and the least suitable enrichment used was mung bean, as shown in Figure 5, since it had the lowest population growth. This study establishes that the population growth rate of *M. micrura* was maximised when enriched with soybean, whereas the lowest growth rate was observed for *M. micrura* enriched with mung bean.

The survival rate of *M. micrura* fed with soybean was (95 ± 4.9%), which is the highest compared to other enrichments, and sesame seed produced the lowest survival rate (78.38 ± 15.76%). For the specific population growth rate, soybean showed the highest result (0.059 ± 0.001%), followed by yeast (0.052 ± 0.002%), sesame seed (0.048 ± 0.002%), and mung bean (0.025 ± 0.004%). The results are shown in Table 1. The survival rate of each treatment is relatively similar, especially for sesame seed and yeast, while mung bean gave the least favourable result, and the results show a significant difference between each treatment ($P < 0.05$).

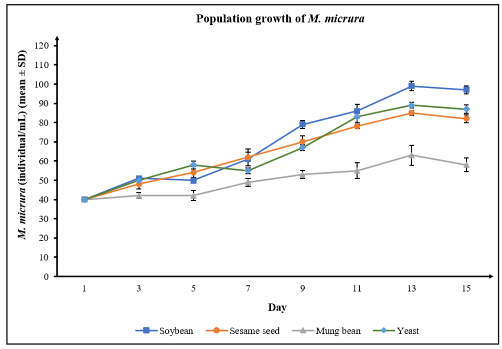

Figure 5. The population growth of *M. micrura* fed with different diets

Table 1
Survival rate and specific population growth rate of M. micrura *fed different enrichments*

Diets	Survival Rate (%)	Specific Population Growth Rate
Mung bean	78.38 ± 15.76[a]	0.025 ± 0.004[c]
Soybean	95.00 ± 4.90[b]	0.059 ± 0.001[a]
Sesame seed	86.88 ± 10.40[a, b]	0.048 ± 0.002[b]
Yeast	90.75 ± 9.05[a, b]	0.052 ± 0.002[b]

Note. All values are mean ± standard deviation (n=3). *Small letters indicate significant difference between treatments

The net reproduction rate (Ro) of *M. micrura* fed with soybean was (9.87 ± 3.78%), and it was comparatively equal to that of yeast (9.20 ± 2.8%). The same can be said of the gross reproduction rate, where soybean (2.67 ± 0.42%) has the best result, followed by yeast (2.47 ± 0.42%). Generation time for both soybean (4.78 ± 0.43 days) and yeast (4.68 ± 0.49 days) have insignificantly difference compared to mung bean (9.27 ± 0.45 days), as highlighted in Table 2. Mung bean has the lowest reproduction results.

Table 2
Net reproduction rate, gross reproduction rate and generation time of M. micrura *fed with different types of enrichments*

Diets	Net Reproduction Rate (%)	Gross Reproduction Rate (%)	Generation Time (Days)
Mung bean	2.20 ± 0.92[b]	0.73 ± 0.31[b]	9.27 ± 0.45[a]
Soybean	9.87 ± 3.78[a]	2.67 ± 0.42[a]	4.78 ± 0.43[b]
Sesame seed	6.67 ± 1.67[a, b]	2.00 ± 0.4[a]	5.80 ± 0.75[b]
Yeast	9.20 ± 2.80[a]	2.47 ± 0.42[a]	4.68 ± 0.49[b]

Note. All values are mean ± standard deviation (n=3). *Small letters indicate significant difference between treatments.

Protein and Lipid Content of *Macrobrachium rosenbergii* Post-larvae

Based on the results from Figures 6 and 7, the lipid and protein content in the *M. rosenbergii* body composition was highest in *M. rosenbergii* fed with soybean-enriched *M. micrura,* with the lipid content of 7.52 ± 0.55%, and protein content of 69.20 ± 0.77%. Meanwhile, *M. rosenbergii* fed with mung bean-enriched *M. micrura* had the lowest lipid content (4.38 ± 0.71%), and protein content (63.49 ± 0.44%).

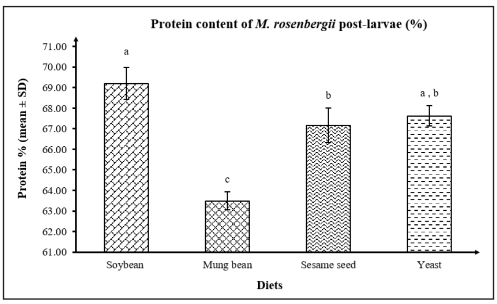

Figure 6. Protein content of *M. rosenbergii* fed with different legume-enriched *M. micrura.* The small letters indicate significant differences between enrichments (*P*<0.05)

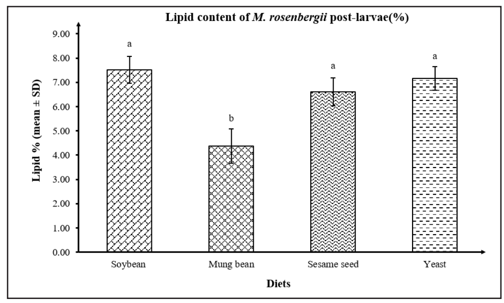

Figure 7. Lipid content of *M. rosenbergii* fed with different legume-enriched *M. micrura.* The small letters indicate significant differences between enrichments (*P*<0.05)

Growth performance and survival of *Macrobrachium rosenbergii* post-larvae

After 30 days of culture, specific growth rate and survival rate of *M. rosenbergii* post-larvae were calculated and presented in Table 3. The highest specific growth rate was obtained when the post-larvae were fed with soybean-enriched *M. micrura* (5.73 ± 0.21%) compared to other diets, while the lowest was obtained when fed with mung bean-enriched *M. micrura.* The highest survival rate was obtained in soybean-enriched *M. micrura* (87.67 ± 2.52%), followed by yeast-enriched *M. micrura* (86.33 ± 2.31%), sesame seed-enriched *M. micrura* (84.33 ± 1.53%), and mung bean-enriched *M. micrura* (78.33 ± 1.53%).

From the results obtained during the 30-day culturing period, the survival rate and specific growth rate of *M. rosenbergii*, when fed with four types of enriched Moina, exhibited discernible differences. *M. rosenbergii* fed with soybean-enriched *M. micrura* demonstrated the highest values for both survival rate (93.67 ± 1.53%) and specific growth rate (5.73 ± 0.21%), suggesting positive effects on the growth and survival of *M. rosenbergii* due to the soybean enrichment. This finding aligns with a study by Suhaimi et al. (2022a), which reported excellent growth performance in tilapia fry fed with *Moina macrocopa* enriched with rice bran and soybean, particularly in terms of weight gain.

Table 3
Specific population growth rate and survival rate of M. rosenbergii *fed with different enriched* M. micrura

Diets	Specific Growth Rate	Survival Rate (%)
Mung bean	3.84 ± 0.23^{c}	78.33 ± 1.53^{b}
Soybean	5.73 ± 0.21^{a}	87.67 ± 2.52^{a}
Sesame seed	4.85 ± 0.12^{b}	84.33 ± 1.53^{a}
Yeast	$5.26 \pm 0.37^{a, b}$	86.33 ± 2.31^{a}

Note. All values are in mean ± standard deviation (n=3). *Small letters indicate significant differences between treatments

DISCUSSION

The composition of protein and lipid in the feed utilised in this study unequivocally influences the corresponding protein and lipid content within Moina. Soybean, closely followed by yeast, exhibits heightened levels of protein and lipid, thereby augmenting the nutritional profile of *M. micrura* and concurrently contributing to the enhancement of their growth and reproductive development. The absorption of protein in Moina is high in soybean feed, with 73.92 ± 0.13% of protein level in enriched Moina, despite its lower protein content of 39.47 ± 1.27% compared to yeast with 51.41 ± 2.52%.

Research suggests that the complex composition of yeast cell walls, abundant in β-glucans and mannoproteins, could inhibit protein bioavailability. A study indicates that these components may decrease nutrient absorption in animals. In contrast, soybean meal possesses a more digestible protein composition and a well-balanced amino acid profile, resulting in improved protein absorption. This indicates that, despite yeast's high protein content, its structural complexity may restrict its efficacy as a protein source in comparison to soybean meal (Morales-López et al., 2009). Nevertheless, it is imperative to conclude that the feed employed for enriching *M. micrura* must align with the zooplankton's consumable suitability.

The absorption and fat content in *M. micrura,* as in Figure 4, do not significantly vary among cultures with feeds of differing fat content. The lipid content in Moina exhibits minimal variation when cultured with feeds of varying lipid content. This phenomenon can be related to Moina's selective absorption and metabolic regulation mechanisms, which sustain a relatively stable lipid composition despite fluctuations in dietary lipids.

A study by Shidik et al. (2021) found that although the overall lipid content in *Moina* sp. was largely stable across various feed treatments, the composition of specific fatty acids fluctuated based on the diet. For example, Moina cultivated with biofloc-based feeds demonstrated elevated levels of saturated fatty acids, while those nourished with Chlorella-based diets showed enhanced polyunsaturated fatty acids. This indicates that although Moina can adjust its fatty acid composition according to available dietary sources, its total lipid content remains consistent, underscoring the species' capacity to effectively regulate lipid absorption and metabolism.

This discrepancy suggests potential limitations in meeting the dietary requirements of *M. micrura* or its suitability as a feed source. Furthermore, there appears to be a dearth of research on zooplankton enrichment with mung bean in comparison to soybean and yeast, emphasising the need for comprehensive investigations. An additional insight emerges from a study by Fouzi et al. (2021) indicating that the combination of fishmeal and dry yeast exhibited the highest density across all sample days, underscoring the importance of specific enrichment combinations. To optimise the nutritional content in the bodies of *M. macrocopa*, it becomes imperative to augment their diet with feeds of high nutritional value, as emphasised by Suhaimi et al. (2022b).

Zooplanktons fed with modified diets have been proven to have excellent biochemical properties and enhance larval appetites and feeding (Olivotto et al., 2011). Similar observations were reported by El-Khodary et al. (2020), who identified that a diet primarily composed of soybeans led to the highest population density in *Cyclops abyssorum divergens*. Hakima et al. (2013) further supported these findings, highlighting that manipulating the quality and type of feed can significantly enhance the population growth of *Moina* sp., with soybean emerging as the most conducive for cultural development. The implications of this research extend to the broader context of aquaculture hatcheries, emphasising that the sustainability and successful production of live feed hinge upon the specific type of feeding utilised.

This study observed that the growth of *M. micrura* decreased significantly in mung bean-fed enrichment, presumably due to the inferior nutritional value of mung beans relative to other enrichments. Furthermore, mung beans are rich in carbohydrates but may be deficient in essential fatty acids and proteins required for optimal *Moina* reproduction and growth. Mung beans also possess anti-nutritional compounds, including tannins, phytic acid, and trypsin inhibitors, which may hinder digestion and nutrient absorption (Cheng & Langrish, 2025). Higher specific population growth rate (r-values) might result from reduced generation times or enhanced fecundity (Khoo et al., 2013).

Notably, enriched *M. micrura* with the highest protein and lipid content also demonstrated the highest observed survival rate among the various feeds examined in the study. According to Azani et al. (2022), enhancing zooplankton can be achieved by introducing different types and levels of nutrients into their diets, a notion supported by both Sarma et al. (2020) and Damayanti et al. (2020).

Concerning the reproductive aspects of *Moina* sp., it is evident that soybean-enriched *M. micrura* exhibits superior outcomes for net reproduction rate, generation time, and gross reproduction rate. This underscores the efficacy of soybean as the most suitable enrichment for enhancing the reproduction of *M. micrura.* In alignment with the research conducted by Azuraidi et al. (2013), tropical Cladocera, including

Moina sp., demonstrate a rapid maturation process, reaching maturity as early as day 3, consequently leading to heightened reproductive rates. The reproductive success of Cladoceran offspring is notably impacted by the introduction of various enrichment types, as highlighted by Azani et al. (2022).

Crucial nutrients such as protein and lipids play pivotal roles in the reproductive processes of *M. micrura.* In accordance with Fink et al. (2011), elevated levels of the diet's nutrient content significantly influence reproduction process in cladocera. Proteins are essential for egg development and overall reproductive success, while lipids serve as an energy source and are vital for the formation of reproductive tissues. Feeds with suitable protein and lipid contents contribute to a high reproduction rate, with soybeans proven to be the most effective enrichment for manipulating the reproduction rate of *M. micrura* compared to other enrichments utilised in this study. The different ways soybean and mung bean enrichment affect reproduction highlight the importance of recognising the specific nutrients in various legumes. This understanding is crucial in meticulously developing feeds that provide the best conditions for zooplankton culture in aquaculture.

Upon subjecting *M. rosenbergii* to varying enriched *M. microra* diets, discernible differences in the protein and lipid composition of *M. rosenbergii* were observed but the protein and lipid profiles of the post-larvae are comparable as the protein and lipid profiles of Moina. When it consumes Moina as feed, their protein and lipid profiles may closely mirror those of Moina due to nutrient assimilation. Research indicated that the dietary habits of aquatic organisms substantially influence their biochemical composition.

Protein and lipid sources in a prawn's diet can influence their body composition, encompassing cholesterol levels and fatty acid profiles. Research on juvenile Pacific white prawns (*Litopenaeus vannamei)* indicated that substituting fish meal and fish oil with soybean meal and soy oil did not impact growth performance but decreased body cholesterol levels (Cheng & Hardy, 2004). Notably, those fed with soybean-enriched *M. micrura* exhibited the highest values for both protein and lipid content. Protein and lipid are pivotal nutrients known for their substantial roles in larval development (Radhakrishnan et al., 2020). As articulated by Bhavan et al. (2010), protein is imperative for the formation, maintenance, and normal function of bodily tissues in *M. rosenbergii*. Furthermore, lipids serve as the primary energy source through metabolic processes.

Although the utilisation of zooplankton, particularly *Artemia*, is prevalent in larval production, alternative zooplankton such as *M. micrura* and copepods can serve as viable substitutes, exerting an influence on the nutritional composition of the cultured species (Joshua et al., 2022). Previous studies have reported an elevation in protein, carbohydrate, and lipid levels in *Catla catla* fry when exposed to various live feeds, including cyclopoid *Thermocyclops decipiens* and cladoceran *M. micrura* (Manickam et al., 2020). The use of enriched *M. micrura* as a live fish food has been demonstrated to enhance the nutritional content of *M. rosenbergii*, affirming its potential as a valuable dietary component in aquaculture practices.

A study by Huang et al. (2017) demonstrated analogous patterns in growth and physiological development when dietary fishmeal was substituted with soybean meal in their diet composition. While the soybean treatment yielded the highest growth performance and survival rate for *M. rosenbergii*, it is noteworthy that the yeast and sesame seed treatments also produced favourable outcomes in terms of both survival rates and specific growth rates. The growth rate assumes a pivotal role in shaping the survival outcomes of *M. rosenbergii*. A superior growth rate is typically indicative of heightened overall robustness and resilience within *M. rosenbergii*, potentially resulting in elevated survival rates.

In a study conducted by Sukri et al. (2016), heightened nutritional formulations were observed to correlate with increased survival rates and enhanced growth of *M. rosenbergii*. Notably, *M. rosenbergii* subjected to a diet enriched with soybean-fed *M. micrura* exhibited the highest survival rate, underscoring the efficacy of utilising highly nutritious feeds to augment the survival of the cultured species. The disparities in growth and survival rates of *M. rosenbergii* post-larvae can be attributed to divergent preferences in nutritional quality, feeding habits, and digestive dynamics.

The early post-larval phases of *M. rosenbergii* are notably influenced by the types of feeds provided, and the nutritional enrichment of *M. micrura* prior to fish larvae feeding can be effectively achieved through diverse enrichment strategies (Aziz et al., 2023). Given that the post-larval phases of *M. rosenbergii* are predominantly predatory and reliant on live food sources (Das et al., 2007; Martin et al., 2006; Sornsupharp et al., 2013), the observed variations in growth and survival underscore the critical importance of tailored nutritional strategies during these developmental stages.

CONCLUSION

This study concluded that the enrichment of *M. micrura* with soybean significantly improved its nutritional profile, resulting in enhanced growth and survival rates of *M. rosenbergii* post-larvae. The enrichment positively affected the development and reproduction of *M. micrura,* thereby confirming the effective transfer of nutrients within the food chain. The outcomes achieved the research objectives by confirming the advantages of nutritional enhancement in live feed. The results indicate the need for additional investigation into plant-based enrichments and provide practical insights for enhancing larviculture protocols in aquaculture.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTION STATEMENTS

AHS conducted the research and prepared the manuscript. AA assisted with the research and manuscript preparation. HJL, NAH, and SRMY contributed to the study design and manuscript revision. TPA and ASM provided technical input and contributed to the critical review of the manuscript. NWR conceived and supervised the study and critically revised the manuscript. All authors read and approved the final manuscript.

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