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The Effect of *Centella asiatica*-Supplemented Diets on Larval Performance and Production of *Macrobrachium rosenbergii* (De Man, 1879)

Riyad Taguemount[1], Jarunan Pratoomyot[2], Andrew P Shinn[3], Hassan Ibrahim Sheikh Mohamed[1], Rasina Rasid[1]*

[1]Faculty of Fisheries & Food Science, Universiti Malaysia Terengganu (UMT), 21300 Kuala Terengganu, Malaysia
[2]Institute of Marine Sciences, Burapha University, 20131 Chonburi, Thailand
[3]INVE (Thailand), 471 Bond Street, Bangpood, Pakkred, Nonthaburi 11120, Thailand

***Corresponding Author: rasinarasid@gmail.com**

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ABSTRACT

The cultivation of *Macrobrachium rosenbergii* in freshwater prawn farming has globally expanded; however, it still faces challenges during the larval phase due to limited availability of high-quality seeds, which reduces production. Live *Artemia nauplii* are traditionally used, but they may not provide sufficient nutrition. *Centella asiatica* (gotu kola powder, GKP) is gaining attention for its potential growth-promoting properties. This study examined the impact of GKP-supplemented diets on larval development and growth. Larvae were initially fed *Artemia* until stage VI, then transitioned to a combination of *Artemia* and GKP-supplemented egg custard diets with varying GKP inclusion rates (0-0.8%). While no significant differences were observed in survival rate, metamorphosis rate, final production, or post-larvae (PL) per liter, the GKP-fed experimental groups performed better overall. Control diet-fed larvae were heavier than those in the 0.8% GKP-fed group, while the 0.2% GKP-fed group showed faster PL appearance. Incorporating *C. asiatica* powder at 0.2% yielded the best results, while the 0.8% GKP-fed group showed the lowest performance. Low GKP inclusion has the potential to support more efficient and cost-effective production methods in aquaculture. Further research should explore lower dosages over longer periods and across different age groups.

INTRODUCTION

The cultivation of *Macrobrachium rosenbergii*, commonly known as the giant freshwater prawn, has seen substantial growth globally, with an annual increase of 6.63% from 2018 to 2022, reaching 337,449 tonnes **(FishStatJ, 2024)**. Despite its potential, the expansion of freshwater prawn farming in Asia faces challenges due to costly seed production, which is primarily reliant on imported *Artemia*. Larvae initially depend on live *Artemia nauplii*, which may not provide sufficient nutrition. In the hatchery of *M. rosenbergii*, larvae primarily depend on live *Artemia nauplii*, which are essential during

the early stages (I to V). As the larvae progress to stage VI and beyond, when their gut develops further and enzyme activities increase, live diets are gradually supplemented with or partially replaced by egg custard-based diets. This enables the larvae to digest and benefit from the additional nutrients provided by prepared feed **(Yong *et al.*, 2018)**.

Nutrition plays a crucial role in seed production, and poor feed quality can impede growth and larval development. Plant-based additives, rich in bioactive compounds, have gained attention for their growth-promoting and immune-boosting properties in aquaculture **(Reverter *et al.*, 2017)**. Gotu kola (*Centella asiatica*), contains various bioactive compounds, including asiatic acid, madecassic acid, and saponins, along with essential nutrients like proteins, lipids, and vitamins **(Seevaratnam *et al*., 2012)**. *Centella asiatica* contains approximately 13.10 ± 1.07% moisture, 8.35 ± 1.28% protein, 1.20 ± 0.10% lipid, 17.00 ± 1.87% fiber, 16.5 ± 0.45% ash, and 43.81 ± 0.70% carbohydrate **(Ogunka-Nnoka *et al.*, 2020)**. Gotu kola also contains fatty acids, amino acids, and vitamins **(Pal & Pal, 2016; Ogunka-Nnoka *et al*., 2020)**. In addition, it contains nutrient inhibitors like phytates, as well as potentially harmful substances used in pond preparation, such as tannin **(Chong & Aziz, 2011)** and saponin **(Roy, 2018)**. Given the presence of numerous bioactive compounds in *C. asiatica*, it has been employed as a feed additive in aquaculture to achieve various effects, including improved digestion, pathogen resistance, and stimulation of non-specific immune responses **(Awad & Awaad, 2017)**, appetite enhancement **(Kawamura *et al*., 2019)**, and growth stimulation **(Tadese *et al*., 2022)**. The occurrence of these bioactive compounds within *C. asiatica* highlights their capacity to impact physiological processes, offering valuable nutritional and therapeutic possibilities in *M. rosenbergii* juveniles **(Salini & Thomas, 2018)**. For example, the levels of inclusion of plant products and plant extraction in *M. rosenbergii* juvenile diet were in the range of 250-750mg/ kg diet **(Salini *et al*., 2014)**. Additionally, *C. asiatica* was incorporated at 0.2% in the diet **(Salini *et al*., 2013)**.

Despite the potential benefits, there is limited research on the effectiveness of *C. asiatica* on *M. rosenbergii* larvae, particularly in the larval zoeal stages. This study aimed to evaluate the effects of gotu kola powder (GKP) supplemented diets on the performance and production of *M. rosenbergii* larvae, offering insights into improving larval feed quality and seed production in the aquaculture industry.

MATERIALS AND METHODS

1. Source of animals

Larvae of *M. rosenbergii* were obtained from ovigerous females in the Manir River (05º 17′ 39.2″ N; 103º 05′ 23.1″ E), Kuala Terengganu, Terengganu. Following their capture, they were transported to the Faculty of Fisheries and Food Sciences (FPSM), University of Malaysia Terengganu (UMT), Terengganu, Malaysia. Each female was

individually housed in an 80-liter black plastic tank containing 11.16 ± 0.60ppt water upon arrival, approximately 15-20 days prior to hatching, and acclimated to hatchery conditions. Gravid females were fed twice a day at 9:30 and 18:00 on a dry pelleted feed consisting of 40% protein (5% body weight day-^{1}). Newly hatched larvae were harvested 24 hours post-hatching and transferred to 130-liter experimental tanks.

2. Diet preparation

The experimental diets, detailed in Table (1), were prepared using the following inclusions of GKP (100% pure powder sourced from Best Naturals, Kenilworth, NJ): 0.2% (GKP1), 0.4% (GKP2), 0.6% (GKP3), and 0.8% (GKP4). Dry ingredients were weighed; eggs and fish oil were added, and then blended until smooth. The mixture was then steamed for 10-15 minutes to form an egg custard. After cooling, this outcome was stored at -18°C until required.

Table 1. The proximate composition of the experimental diets presented to the *Macrobrachium rosenbergii* larvae. Ingredients are given as % and are the mean ± SD of three replicates

Ingredients	Control	GKP 1	GKP 2	GKP 3	GKP 4
	GKP (0%)	GKP (0.2%)	GKP (0.4%)	GKP (0.6%)	GKP (0.8%)
Chicken egg	47	47	47	47	47
Milk powder[1]	20	20	20	20	20
Rolled oats[2]	10	10	10	10	10
Fishmeal[3]	8	8	8	8	8
Fish oil[4]	5	5	5	5	5
Diatomaceous earth[5]	2	2	2	2	2
CMC[6]	2	1.8	1.6	1.4	1.2
GKP[7]	--	0.2	0.4	0.6	0.8
Catalyst[8]	3	3	3	3	3
Mixed vitamins[9]	1.5	1.5	1.5	1.5	1.5
Mixed minerals[10]	1.5	1.5	1.5	1.5	1.5
Proximate analysis (dry wt)					
Moisture	53.70±0.11	53.10±1.15	53.13±2.12	53.01±1.38	52.10±1.97
Protein	53.19±1.34	52.14±1.03	51.66±1.82	51.89±1.59	52.69±1.19
Lipid	34.50±1.41	34. 46±1.48	34.90±1.11	33.99±1.88	33.22±1.28
Ash	09.61±0.12	10.79±0.95	10.81±1.30	10.99±1.56	10.95±1.42
Fibre	01.38±0.01	01.39±0.01	01.39±0.01	01.39±0.01	01.39±0.01
NFE	01.32±3.60	01.22±1.92	01.24±1.13	01.74±1.40	01.75±1.78

Data is mean ± SD; NFE = Nitrogen-Free Extract, including fiber.

Abbreviations: CMC = carboxymethylcellulose; GKP= gotu kola powder. [1] Nestle Products Sdn. Bhd., Petaling Jaya, Kuala Lumpur, Malaysia; [2] Scc Marketing (M) Sdn Bhd., Puchong, Selangor, Malaysia; [3] TripleNine, 700 g kg^{-1} crude protein; [4] TripleNine, Denmark 100% marine oil; [5] Global Agora Resources, Bandar Pinggiran Suban 40150 Shah Alam, Malaysia; [6] D Chemie Chemical Supplie, Skudai, Johor, Malaysia; [7] GKP = Gotu kola powder (Best Naturals, Kenilworth, NJ); [8] Barkath Foods Sdn. Bhd, Seberang Perai Tengah, Malaysia; [9] Vitamin premix contained (as g/kg) vitamin A, 50; vitamin D3, 10; vitamin E, 130; vitamin K3, 10; vitamin B1, 10; vitamin B2, 25; vitamin B6, 16; vitamin B12, 0.1; niacin, 20; pantothenic acid, 50; folic acid, 8; biotin, 0.5; anti-caking agent, 20 (DSM Nutritional Products (Thailand) Ltd, Chonburi, Thailand); [10] Mineral premix contained (as g/kg) copper, 7.5; iron, 125; manganese, 25; zinc, 125; cobalt, 0.5; iodine, 0.175; selenium, 0.3; anti-caking agent, 10 (DSM Nutritional Products (Thailand) Ltd., Chonburi, Thailand); NFE = Nitrogen-Free Extract.

3. Culture system

A recirculation system comprising fifteen rectangular fiberglass tanks, each holding 150L, was utilized for the feed trial. Each tank contained 130L of brackish water (average salinity of 12.7 ± 0.52ppt). Triplicate tanks were assigned to each diet randomly. Tanks were stocked at a density of 60 larvae/L, a total 7,800 larvae/tank. The larval rearing system included a submerged biological filter with four chambers. Water flowed from the culture system to the biological filter via gravity, with an air-lift pump returning filtered water to the rearing tanks, achieving a turnover rate of 24 times/day. Approximately 10% of the water was replaced daily.

4. Feeding trials and analytical procedures

Five feeding regimes were employed, each offering varying amounts of GKP to the prawn larvae. The feeding schedule included providing nauplii of the Great Salt Lake strain of *Artemia franciscana* (Aquatic Artemia Cysts^{TM}, USA) until stage V. prawn larvae were fed *Artemia* nauplii daily at 10:00, 14:00, 18:00, and 23:00, ranging from 1 to 5 nauplii/mL. Upon reaching stage VI, larvae received a blend of the relevant GKP-supplemented egg custard diet and *Artemia*. The egg custard diet was dispensed at a rate of 0.2-0.5g per 1000 larvae three times daily, adjusted based on larval stage and tank population. *Artemia* constituted the final nocturnal feeding.

5. Water quality parameters

Water quality parameters (temperature, pH, salinity, dissolved oxygen) were assessed daily using a YSI multiparameter meter (5908 Cap Membrane Kit, USA). Ammonia and nitrite concentrations were measured weekly with a Model NI-SA (Loveland, CO, USA) test kit. Water quality was maintained daily by siphoning to remove waste and regular 10% water changes. Throughout the trial, temperature averaged 28.7±0.69°C, dissolved oxygen was 6.76 ± 0.56mg/ L, pH was 7.76 ± 0.39, ammonia and nitrite concentrations were 0.12 ± 0.15 and 0.3 ± 0.95mg/ L, respectively.

6. Larval growth and survival

The experiment concluded upon 95% larval transition to PL (days recorded). Survival rates were determined by counting all larvae and PL in each tank. Fifty PL and larvae were randomly chosen for total length (TL) measured using digital calipers (±0.1 cm), while weight (Wt) was measured using a high-precision balance (AT21 Mettler Toledo, Inc., Shanghai, China).

The calculation formulas used in this study followed those of **Taguemount *et al.* (2024)**:

- Final survival (S_f, 100%) = 100× (N_T /N_0). Where N_T = final number of larvae / PL; N_0 = initial number of larvae.
- Final production (L+PL/L) = final number of larvae and PL per liter.

- Metamorphosis rate (Morph) = (final amount of PL/number of stocked larvae) × 100.

7. Chemical analysis of the diets

The nutrient composition of the experimental diets was assessed via proximate analysis Association of Official Analytical Chemists **(AOAC, 1995)**. The analysis was conducted at the General Quality and Analysis Laboratory, FPSM, UMT. Moisture was determined by drying samples (2g) at 105°C for 24 hours. Ash was measured by the incineration of samples at 600°C for 6 hours using a muffle furnace (Gallenkamp). Crude protein was determined using the Kjeldahl method using a Kjeltec Autoanalyser (FOSS KT200 Kjeltec^{TM}). Crude lipids were extracted using petroleum ether (boiling point 40–60°C) via the Soxtec^{TM} (FOSS Tecator). Nitrogen-free extract (NFE) was calculated by subtracting protein, lipid, and ash from 100.

8. Statistical analysis

One-way analysis of variance (ANOVA) was conducted to detect significant variations ($P<0.05$) among the treatments (SPSS version 26.0). Duncan's multiple range *post-hoc* test was employed to identify specific treatments that differed significantly. Quadratic regression analysis was performed to examine the relationship between dietary treatments and performances.

RESULTS

1. Larval performance

The experimental diets with varying rates of GKP inclusion (Table 1) had consistent levels of moisture, crude protein, crude lipid, ash, and NFE (including fiber). Biometric data of PL under various dietary treatments are summarized in Table (2). Of the *M. rosenbergii* larvae performance indicators, such as wet weight, total length, survival rate, and final production (larvae + PL/L, and PL/L), the only statistically significant difference ($P<0.05$) was observed in the wet weight of larvae fed the control diet compared to those fed the GKP4 (0.8%) diet. Among the GKP diets, larvae fed GKP1 (0.2%) and GKP4 (0.8%) diets exhibited the highest and lowest performances, respectively. Specifically, *M. rosenbergii* larvae fed the control diet had significantly greater wet weight (9.34 ± 0.17mg) ($P<0.05$) compared to those fed the GKP4 diet (7.40 ± 0.98mg) supplemented with 0.8% GKP. No statistically significant differences were observed in wet weight between larvae fed the control diet and those fed diets supplemented with 0.2%-0.6% GKP (i.e., GKP1-3), nor among any of the GKP-supplemented diets (i.e., GKP1-4; $P>0.05$) (Table 2). Similarly, no significant difference ($P>0.05$) was found in total length of larvae between the experimental groups, ranging from 7.48 ± 0.72 to 8.42 ± 1.15mm, although those in the control group were the largest. Though no significant differences were observed in survival rate (55.83 ± 4.70% to 68.38 ± 2.60%), metamorphosis rate (52.83 ± 4.69% to 64.97 ±

2.47%), final production (larvae + PL/L) (33.50 ± 2.82 to 41.03 ± 1.65), or PL/L (31.7 ± 2.82 to 38.98 ± 1.48%), larvae fed diets containing GKP showed slight improvements compared to those fed the control diet (Table 2). Quadratic regression analysis of wet weight (wt) revealed that as GKP percentage increased, wet weight decreased. Conversely, low levels of GKP inclusion (0.2%) showed trends toward improving final production, while high levels of inclusion (0.8%) indicated a trend toward delaying these performances.

2. Larval development

Similarly, no significant differences were observed in the metamorphosis rate (52.83 ± 4.69% - 64.97 ± 2.47%) or in the time to PL 12 (31-33 days). The metamorphosis of larvae fed diets containing GKP showed a slight improvement compared to those fed the control diet (Table 2 & Fig. 1).

Table 2. Performance parameters of the *Macrobrachium rosenbergii* larvae in the experimental larviculture feeding the diets containing different inclusions of *Centella asiatica*, gotu kola powder (GKP). Values are the mean ± SD of three replicates

Performance	%GKP				
	0	0.2	0.4	0.6	0.8
Wt (mg)	9.34±0.17^{a}	8.86±0.41^{ab}	8.15±0.83^{ab}	8.99±1.07^{ab}	7.40±0.98^{b}
TL (mm)	8.42±1.15	7.48±0.72	7.54±1.02	7.90±0.94	8.05±1.33
Survival (%)	55.83±4.70	68.38±2.60	61.32±11.09	62.54±5.04	56.23±12.87
Metamorphosis rate (%)	52.83±4.69	64.97±2.47	58.29±10.54	59.42±4.79	53.42±12.23
95% production (L+ PL/L)	33.50±2.82	41.03±1.65	36.79±6.66	37.53±3.02	33.74±7.72
Number of PL/L	31.70±2.82	38.98±1.48	34.96±6.32	35.65±2.87	32.05±7.34
Raring period (d)	31±0.01	33.00±0.01	33.00±0.01	33.00±0.01	33.00±0.01

Values within the same column with different letters are significantly different (p<0.05). d=day; L+PL/L= larvae + post-larvae/litre; Morph= metamorphosis; PL12=time to complete metamorphosis; PL/L= post-larvae/litre; TL= total length; Wt= weight.

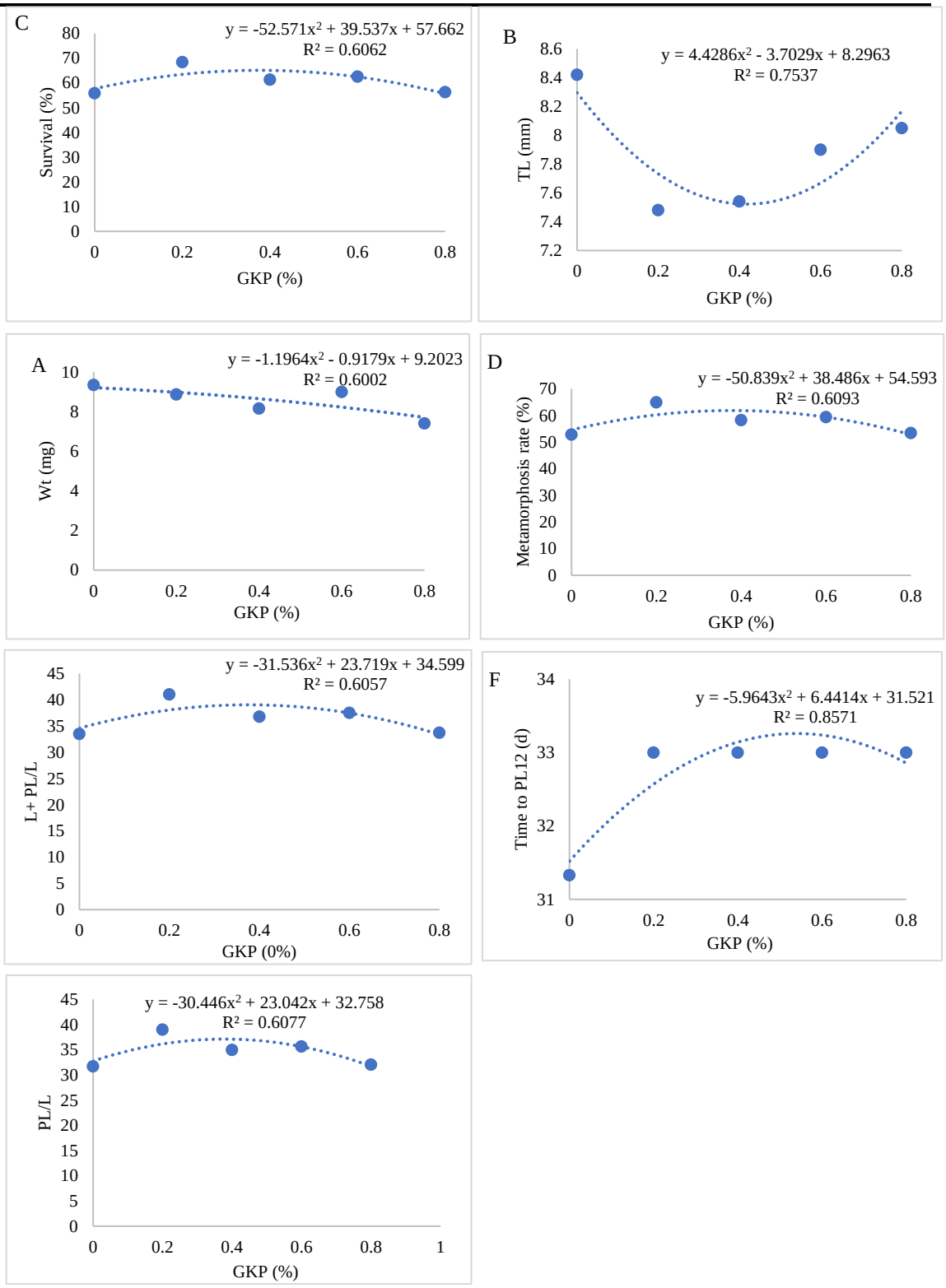

Fig. 1. Quadratic estimation of the optimal level of percentage of gotu kola powder (GKP) at 0.0, 0.2, 0.4, 0.6 and 0.8% inclusion that maximises the growth and production of *Macrobrachium rosenbergii* larvae

DISCUSSION

1. Larval growth and production

The trials conducted on post-larvae and juveniles found that plant products benefited growth and feed utilization in *M. rosenbergii* **(Liu *et al.*, 2010; Radhakrishnan *et al.*, 2014; Salini *et al.*, 2014; Kaleo *et al.*, 2019)**. In this study, the final wet weight of larvae decreased with increasing GKP inclusion. Notably, those on the 0.8% GKP diet had the lowest final weight, which was statistically significantly different from the control diet ($P < 0.05$). This suggests a limitation in the ability to utilize the GKP product at higher levels (Table 2 & Fig. 1). *Centella asiatica* contains both nutrients and antinutritional substances, such as tannin **(Chong & Aziz, 2011)**, saponin **(James & Dubery, 2011)**, and phytate **(Ogunka-Nnoka *et al.*, 2020)**. In the marine environment, saponins serve as kairomones - chemical cues that play a role in attracting symbionts to their respective hosts **(Caulier *et al.*, 2013)**. Saponins enhance nutrient digestibility and absorption **(Acosta *et al.*, 2019)**, potentially influencing larvae performance. The inclusion of GKP at higher concentrations, such as 0.8%, may expose larvae to increased antinutritional substances, inhibiting feed utilisation and resulting in lower final weight and total length. This study suggests that inclusion of *C. asiatica* at low concentrations can affect feed attraction, consumption, and the developmental progress of *M. rosenbergii* larvae. It has been noted that higher dosages of GKP in the diet might alter feed texture and taste, potentially impacting feed utilization **(Sarker *et al.*, 2021)**. Careful consideration of GKP inclusion levels is, therefore, crucial to optimize *M. rosenbergii* larvae growth.

Macrobrachium rosenbergii undergoes long larval stages and cultivation periods. According to **Deru (1990)**, yolk reserves are depleted by stage V, and artificial diets are then fed from stage VI to metamorphosis. During larval development, peak trypsin and esterase activities occur at stage I and V-VI, while α-amylase activity is initially low, sharply increasing around stage VII-VIII, peaking at stage X-XI. This suggests early-stage caridean larvae are exclusively carnivorous **(Kamarudin *et al.*, 1994)**. Amylase predominates over proteases in adult *M. rosenbergii* **(Lee *et al.*, 1980)**. Increasing amylase activity in later larval stages indicates an improved ability to digest plant feed ingredients near the PL stage. *Macrobrachium rosenbergii* larvae fed GKP diets exhibit lower wet weight and total length than those on the control diet, likely due to insufficient amylase levels to hydrolyse plant ingredients. Thus, the age of the tested animals, digestive system development, and enzyme functionality influence the limited utilisation of GKP in this study.

Macrobrachium rosenbergii larvae fed a diet containing 0.2% GKP showed higher survival rates and final production compared to the control diet, though not statistically

significant (Table 2). Survival rates ranged from approximately 56 to 68%, with the 0.2% GKP-fed group performing the best, followed by 0.4-0.6% GKP-fed groups. Quadratic regression analysis indicated an optimal GKP inclusion at 0.2%. At a density of 60 larvae per liter, survival rates were higher compared to a combination of *Artemia* and egg custard at an initial density of 50 larvae per liter, which had a 30% survival rate **(Islam & Ahmed, 2000)**. Survival rates in larvae fed 0.2-0.6% GKP diets ranged from 61-68%, while control (0% GKP) and 0.8% GKP diets had rates around 56%. These rates were superior to those reported for larvae fed diets consisting solely of *Artemia nauplii* at rates of 5, 10, and 20 per mL, which ranged from 52.7 to 59.1% **(De Aviz *et al*., 2018)**. Similarly, final productivity ranged from 32-39%, with larvae fed 0.2% GKP diets showing the highest production, followed by 0.4-0.6% GKP diets. These results align with larvae fed *Artemia nauplii* alone, which showed productivities ranging from 30.1 to 40.0% **(De Aviz *et al*., 2018)**. Overall, GKP inclusion levels up to 0.2% did not negatively impact *M. rosenbergii* larvae survival or production, with no additional benefits observed beyond this threshold.

The effectiveness of plant additives depends on dosage, duration, and administration route. High doses can hinder growth rates and feed intake in fish **(Awad & Awaad, 2017)**. *Macrobrachium rosenbergii* larvae fed 0.2% GKP showed slight growth improvement without significance (Table 2 & Fig. 1), suggesting benefits at low levels. Previous studies found low plant inclusion levels enhanced growth in shrimp and prawn species **(Bhavan *et al*., 2011; Bhavan *et al*., 2020)**. For instance, optimal *Quillaja* saponin inclusion at 0.36g/ kg improved growth and immune responses in *P. monodon* **(Jumah *et al*., 2020)**. Inclusion of 0.05% *C. asiatica* boosted growth in *M. rosenbergii* **(Salini *et al*., 2014)**. Anthraquinone extracts at 0.10-0.20% promoted *M. rosenbergii* growth **(Liu *et al*., 2010)**. Notably, GKP levels in this study exceeded those of previous investigations, but were similar to some **(Radhakrishnan *et al*., 2014)** and lower than others **(Kaleo *et al*., 2019)**. Origin, sources, and utilization methods of plant products also affect dosage. Given the favorable results at 0.2% GKP, further research should explore lower GKP levels in diets.

2. Larval development

The findings of this study suggest that the addition of GKP to diets, ranging from 0.2 to 0.8%, does not significantly impact the development of *M. rosenbergii* larvae. Larvae fed on these experimental diets took approximately 31 to 33 days to reach 95% metamorphosis by PL12 stage. However, this duration can vary depending on several factors, such as water temperature, food quality and availability, water conditions, the genetic lineage of the breeding stock, and, importantly, the proficiency of the hatchery operator **(Nandlal & Pickering, 2005)**. **Bart and Yen (2003)** observed that the duration for 100% metamorphosis was 36 days; **Nandlal and Pickering (2005)** noted that *M. rosenbergii* larvae raised in captivity typically achieved the PL stage within 22 to 35 days.

New (2002) suggested that under water temperatures of 28-31°C, most *M. rosenbergii* larvae should reach PL stage within 25 to 32 days.

CONCLUSION

In conclusion, this three-week study revealed that incorporating *C. asiatica* powder in *M. rosenbergii* larval diets influenced growth, development and seed production. Larvae on GKP diets showed reduced weight and length, whilst those fed on the GKP supplemented diets between 0.2-0.4% inclusion showed better metamorphosis and seed production, especially at 0.2% inclusion. Beyond this, no added benefits were observed, making higher inclusions economically inefficient. Egg custard-enriched diets offer a cost-effective alternative to *Artemia*, enhancing seedling production. Future studies should explore lower dosages of *C. asiatica* across varied age groups and longer experimental durations.

Declaration of interest

All authors declare that there is no conflict of interest to disclose.

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