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Enhancing the Physical Properties of Egg Custard-based Diets through Feed Supplements to Improve the Growth Performance of *Macrobrachium rosenbergii* Larvae

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ABSTRACT

The giant freshwater prawn, *Macrobrachium rosenbergii*, is an important species in freshwater crustacean aquaculture. Traditional larval feeding methods relying on egg custard are challenged by poor buoyancy and significant nutrient leaching. This study aims to enhance the physical properties of egg custard-based diets by incorporating carboxymethyl cellulose (CMC) and buoyancy modifiers (BM, yeast, and baking powder). Three experimental diets were formulated with varying CMC and BM levels (D1: 5% CMC; D2: 2% CMC & 3% BM; D3: 0% CMC & 5% BM) and evaluated on floatability, water stability, and nutrient retention. The study also involved feeding larvae with these diets and assessing growth performance over several weeks. Assessing the physical properties of each diet over 60 min found that D3 was the most buoyant, achieving a flotation rate of 49.24%, while D2 had a rate of only 32.98%, and D1 20.90%. Water stability assessments showed that D1 retained 70.80% stability after 40 min, compared to 69.55% for D2 and 86.61% for D3. The nutrients of nutrient leaching were not too dissimilar: D1 had a protein retention of 89.00%, D2 a retention at 87.60%, and D3 86.61%. The lipid retention of each diet followed a similar trend, with D1 retaining 90.21% of its lipid content, D2 88.49%, and D3 at 84.47%. This study highlights the importance of optimising dietary formulations for *M. rosenbergii* larvae, demonstrating that balancing buoyancy and nutrient retention can lead to improvements in the growth and survival of larval cultures. The study concludes that incorporating CMC and BM can enhance diet quality and larval performance.

Keywords: Aquaculture, feeding strategy, growth performance, *Macrobrachium*, nutrient leaching, water stability

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INTRODUCTION

The aquaculture of the giant freshwater prawn, *Macrobrachium rosenbergii,* is economically significant, ranked as the fourth most important crustacean species, in terms of tonnage, globally (Wahl et al., 2023). In the hatchery phase of production, the larvae are traditionally fed live *Artemia* nauplii and egg custard (Taguemount et al., 2024). While *Artemia* are highly mobile, the egg custard particles do not move, and these must be caught by chance as they pass the prawn larvae; this property of egg custard may potentially hinder the successful growth and survival of larvae (Taguemount, 2025). Although the egg custard, made from chicken eggs and skimmed milk, is easily digestible, its tendency to sink makes it unsuitable for *M. rosenbergii* larvae, which feed in the water column. Additionally, the high moisture content of the egg custard results in low water stability and significant nutrient leaching (Dhont et al., 2010). Typically, shrimp feed contains 10-40% fishmeal and 9-20% soybean meal, though the reliance on fishmeal is declining due to the cost of these ingredients and their sustainability (Kasamechotchung et al., 2025; Nunes et al., 2022). The inclusion of fish and squid oils (3%) enhances feed attraction (Yusoff et al., 2021), while starches (10-20%) serve as energy sources and binders (Munkongwongsiri & Poolsawat, 2024; Tinh et al., 2021). Other dietary ingredients include fibre, vitamins, and minerals (Ayisi et al., 2017; Nesara & Paturi, 2018); effective formulations must consider both the nutritional composition and the physical properties of the diet. Additives such as cellulose (1-3%) help improve buoyancy and stability, while binders like carboxymethylcellulose (CMC) and agar help enhance feed integrity (Ayuhafizah et al., 2024). The incorporation of *Rhizopus oligosporus* mycelium can also enhance structural stability and buoyancy (Nurohmah et al., 2023).

The stability and floatability of the aquatic diet, therefore, are crucial for prawn feed. Ideal feed should maintain buoyancy, settle slowly, and minimise nutrient loss, thereby accommodating the continuous feeding habits of the larvae (Jannathulla et al., 2019). Water stability refers to pellet integrity and minimal disintegration (Karim et al., 2022), while the floatability can help determine the suitability of a diet for a particular feeding strategy (Irungu et al., 2018). The bulk density is closely related to the sinking velocity of feed, with high-density particles sinking faster (Jannathulla et al., 2019). Understanding the sinking and leaching rates is important as this knowledge can lead to optimised feeding regimes (Zaabwe et al., 2020). For the larviculture of *M. rosenbergii*, encapsulation is not a viable strategy for high-moisture feeds (>50%), so a practical approach to this is to include specific ingredients to enhance buoyancy and to reduce nutrient leaching (Aaqillah-Amr et al., 2021).

A wide range of different ingredients and techniques have been explored to improve the physical properties of aquafeeds. Binders, for example, help maintain diet integrity and cohesion when in water (Miladinovic, 2020), with carboxymethyl cellulose (CMC) being

widely recognised as a cost-effective binder that improves pellet stability (Rahman et al., 2021). For *M. rosenbergii*, optimal feed stability has been reported with inclusion rates of 2% to 4% CMC (Aaqillah-Amr et al., 2021; Yusoff et al., 2021). Other method to improve feed buoyancy include incorporating air into the feed matrix, adjusting oil levels, and using upwelling currents during feeding (Kolkovski, 2004). The use of density control agents can reduce feed density, aiding flotation (Kari et al., 2023), while fungal fermentation with oven drying has also been shown to improve water stability and floatability (Sriherwanto et al., 2021). The carbohydrate content of diets can also influence these properties; for example, guinea corn has been reported to outperform maize and wheat in producing floating pellets (Fashina et al., 2019). The combination of wheat offal with yeast has also been shown to provide superior expansion and floatation characteristics (Momoh et al., 2016). Gelatin, CMC, and guar gum have been demonstrated to enhance water stability and growth performance across various species (Ayoola, 2020; Gao et al., 2020).

The use of yeasts and baking powder represents another approach to enhancing pellet buoyancy (Suleiman et al., 2008). Yeast contributes to buoyancy through the production of carbon dioxide (CO_2) during fermentation (Tofalo et al., 2020), while baking powder, a leavening agent, comprised of sodium bicarbonate (baking soda) and an acid component, typically phosphate salts, releases CO_2 on contact with moisture. This release of CO_2 aerates the feed matrix and contributes to its buoyancy (Kollemparembil et al., 2023). Orire and Sadiku (2014) found that diets incorporating 10% of these ingredients, i.e., yeast and baking powder, had a flotation of approximately 70% after 60 minutes. When the inclusion of these was reduced to 5%, 60% of both pellets and flakes remained afloat after 60 minutes, suggesting adequate buoyancy. Standard egg custard particles sink and leach nutrients; therefore, the buoyancy and nutrient retention of the diet needs to be improved if it is to meet the nutritional needs of the *M. rosenbergii* larvae. This study, therefore, investigates the inclusion of CMC and buoyancy modifiers (yeast and baking powder) on the integrity and buoyancy of egg custard-based diets, and on the larviculture performance of larvae.

MATERIALS AND METHODS

Animal Husbandry

Gravid *M. rosenbergii* females (average weight of 38.05 ± 2.87 g) were collected from the Manir River, Terengganu, Malaysia (05°17′39.2″N, 103°05′23.1″E) and transported to the marine hatchery at Universiti Malaysia Terengganu, Malaysia. The berried females were housed individually in 80 L tanks, where the salinity was gradually increased to 12 ppt. The female prawns were fed twice daily using a commercial pelleted feed (40% protein, Gold Coin Pro Series, Indonesia) supplemented with fresh mussels at a rate of 5% body weight per day (i.e., an average daily feed ration of 1.90 g).

After 15 to 20 days of maintenance, the larvae hatched (primarily overnight); these were then counted and collected using a five-point method under low lighting conditions and then transferred to the larval-rearing tanks.

Experimental System and Water Management

The larviculture setup included 18 × 150 L rectangular, dark blue fibreglass tanks connected to a submerged biofilter divided into multiple chambers. Each tank was filled and maintained with 130 L of 12 ± 1 ppt seawater and a porous air stone for aeration. A gravity-fed water system circulated water from the larviculture tanks to the biological filter. An outlet screen with a mesh size of 250 µm was placed at the top of the drainpipe (2.5 mm) to allow water to flow back into the biological filter while retaining the larvae and *Artemia* within the rearing tank. An air-lift pump was used to return the filtered water to the rearing tank. To prevent water overflow, the filter screen was cleaned daily. Any water loss due to evaporation was compensated for by adding more brackish water. Each of the six experimental diets was randomly assigned to triplicate groups of larvae, starting with 7,800 larvae per tank at a stocking density of 60 larvae/L. Daily maintenance involved removing residual feed, dead larvae, and waste before the morning feeding, along with ~10 % daily water changes to maintain optimal water quality. Water quality parameters, temperature, pH, salinity, and dissolved oxygen were monitored using a YSI multiparameter meter (5908 Cap Membrane Kit, USA), with ammonia and nitrite levels recorded using a test kit (Model NI-SA, Loveland, CO, USA). The results showed an average water temperature of 28.94 ± 3.96 °C, dissolved oxygen at 6.23 ± 1.06 mg/L, pH at 8.63 ± 1.7, and salinity at 12.63 ± 1.85 ppt. Total ammonia and nitrite levels were consistently below 0.1 mg/L. A natural photoperiod of at least 12 hours daily was maintained to support larval growth.

Experimental Diets

Two experimental diets (D2 and D3) and a control (D1) were formulated with the following percentages of CMC and BM: D1 (5% CMC & 0% BM), D2 (2% CMC & 3% BM), and D3 (0% CMC & 5% BM). The BM consisted of a 50:50 mixture of yeast and baking powder. To achieve the 3% and 5% BM levels in the diets, yeast and baking powder were added in equal ratios. Therefore, the (CMC: BM) ratios were 5/0 (all CMC), 2/3 (mixed), and 0/5 (all BM) (Table 1). Each dry ingredient was individually weighed and thoroughly mixed. Chicken eggs and fish oil were then added to the mixture and blended using a Pensonic MX-GM 1011 blender for 5 minutes, ensuring complete integration of all the ingredients. The homogenate was then supplemented with activated yeast and baking powder as the buoyancy modifier. This mixture was allowed to rest at room temperature for 30 minutes to allow the yeast to activate. Then, the mixture was placed in a two-tiered stainless-steel steamer and steamed using a portable gas stove (Pensonic PPG-2002N), with the lower containing boiling water, while the upper perforated tier allowed steam to pass through and

cook the egg custard diet. This setup is commonly used for steaming food and represents a healthy cooking method that preserves nutrients (Martinez, 2024). The steaming was done at a temperature range of 75 to 100 °C for precisely 15 minutes. After steaming, the diet was allowed to cool to room temperature before further handling. The prepared diet was refrigerated at 5 °C and used within a few days of preparation to ensure freshness and nutritional integrity. The nutrient content of the experimental diets was analysed according to the methods outlined by the Association of Official Analytical Chemists (AOAC) (1995). Parameters assessed included moisture, ash, protein, lipids, and fibre (Table 1).

Table 1
Composition and nutrient analysis of the formulated experimental diets

Ingredients (g/100g dry weight)	**Control (0% BM)**	**3% BM**	**5% BM**
	D1	**D2**	**D3**
Chicken egg	47	47	47
Milk powder[1]	20	20	20
Rolled oats[2]	10	10	10
Fishmeal[3]	8	8	8
Fish oil[4]	5	5	5
Diatomaceous earth[5]	2	2	2
Carboxymethylcellulose[6]	5	2	0
Mixed vitamins[7]	1.5	1.5	1.5
Mixed minerals[8]	1.5	1.5	1.5
Buoyancy modifier[9]	-	3	5
Proximate composition[10] % dry matter			
Moisture (% of wet weight)	53.05 ± 0.51	52.37 ± 0.16	46.99 ± 2.54
Other Components (% of dry matter)			
Ash	9.2 8 ± 0.08	8.99 ± 0.10	09.13 ± 0.61
Protein	50.14 ± 0.64	50.68 ± 2.45	50.51 ± 1.65
Lipid	37.61 ± 0.33	37.79 ± 2.15	37.86 ± 2.19
Fibre	01.40 ± 0.00	01.40 ± 0.00	01.40 ± 0.00
NFE	01.65 ± 0.22	01.14 ± 0.32	01.10 ±0.18

Note. BM = buoyancy modifier. D1 (control) = 5% CMC & 0% BM; D2 (3%BM) = 2% CMC & 3% BM; D3 (5% BM) = 0% CMC & 5% BM. NFE = Nitrogen-Free Extract

[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, 100% marine oil; [5]Global Agora Resources., Bandar Pinggiran Suban 40150 Shah Alam, Malaysia; [6]D Chemie Chemical Supplie, Skudai, Johor, Malaysia; [7]Vitamin premixes 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 700/437 Chonburi, Thailand; [8]Mineral premixes 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; [9] Taiwan; [10]Seafield Food Ind. Sdn. Bhd, Malaysia, and Barkath Foods Sdn. Bhd, Malaysia

Feeding Trial

From day 2 post-hatch, larvae were initially fed newly hatched *Artemia* nauplii at 1-5 nauplii/mL four times daily (10:00, 14:00, 18:00, and 23:00 h). Starting on day 7 (stage 6 to PL), the diet included *Artemia* nauplii and various formulated egg custard diets: control (5% CMC & 0% BM), D2 (2% CMC & 3% BM), and D3 (0% CMC & 5% BM). Larvae were fed 0.2-0.3 g of the relevant egg custard diet for days 8-15 and 0.3-0.5 g for days 16-28 per 1000 larvae (Murthy et al., 2008). Feed particles were manually sieved (pore size ~ 400 -1000 µm), then mixed with culture water (20 mL), and fed using a Pasteur pipette to ensure larvae could catch feed particles. Feeding of prepared feed occurred three times a day during daylight hours (10:00, 14:00, and 18:00 h), and once overnight at 23:00 hour with *Artemia* nauplii.

Evaluation of the Physical Properties of the Experimental Diets

Morphology of Feed Particles

The surface morphology of the egg custard feed particles was examined using a scanning electron microscope (3rd Generation VEGA SEMs, Tescan, Czechia). For sample preparation, the diets were thoroughly dried at 40°C to remove moisture. Particles of each diet were then mounted on the SEM sample holder using double-sided carbon tape. Subsequently, the samples were sputter-coated with a thin layer of gold (approximately 10-20 nm thick) in an argon atmosphere to enhance electrical conductivity and prevent charging during electron beam exposure (Anas et al., 2008). The surface morphology of each diet was then examined to assess the influence of each inclusion of CMC and BM.

Floatation Test

The floatability of the egg custard feed particles incorporating a buoyancy modifier (BM) was assessed (Equation 1) under the standard rearing conditions for prawn larvae (12 ± 1 ppt salinity; 27 ± 1°C). Formulations tested included D1 (5% CMC & 0% BM), D2 (2% CMC & 3% BM), and D3 (0% CMC & 5% BM). Before testing the properties of each diet, the aeration and water circulation were stopped to accurately measure floatability. Based on a stocking density of 7,800 larvae per tank and a feeding rate of 0.325 g of feed per 1,000 larvae per day, the total quantity of daily feed was calculated to be 2.535 g. Three feeds were given each day (0.845 g per feeding). During each flotation test, 0.0845 g (10% of 0.845 g) of each diet was added to a floating ring representing 10% of the water surface area (300 cm^2 of 3,003 cm^2). The average number of particles in 0.0845 g was 22.33 ± 0.58 for D1, 31.33 ± 1.53 for D2, and 44 ± 1.73 for D3. Each assessment lasted 60 min, with the number of pellets recorded at 10-min intervals across triplicate tanks (18 replicates per feed type).

$$\text{Floatation rate (\%)} = (\text{Final no. of particles afloat}) / (\text{Initial no. of particles afloat}) \times 100 \quad [1]$$

Water Stability and Dietary Nutrient Leaching

To determine the water stability (Equation 2), a 5 g sample of each experimental diet (D1 contained 5% CMC & 0% BM, D2: 2% CMC & 3% BM, D3: 0% CMC & 5% BM) were placed on 0.1 mm mesh nylon filter cloths and immersed in the larviculture tanks (130 L, 12 ± 1 ppt salinity, 27 ± 1°C) containing no prawn larvae. Each mesh was secured to prevent physical damage during immersion. Representative samples of each diet were removed after 10, 20, 30, and 40 min, then air-dried, and weight loss was determined by comparing initial and post-immersion weights (Kovalenko et al., 2002). All measurements were conducted in triplicate. Following immersion, the protein (Equation 3) and lipid content (Equation 4) of each sample of diet was analysed using the Kjeldahl and Soxhlet extraction methods, following AOAC (1995) protocols.

$$\text{Water stability (\%)} = (\text{initial feed weight-dried feed weight (g)}) / (\text{initial feed weight (g)}) \times 100 \quad [2]$$

$$\text{Protein retention (\%)} = (\text{Post-immersion protein content}) / (\text{Initial protein content}) \times 100 \quad [3]$$

$$\text{Lipid retention (\%)} = (\text{Post-immersion lipid content}) / (\text{Initial lipid content}) \times 100 \quad [4]$$

Evaluation of Larval Development and Growth Parameters

The larval development of the *M. rosenbergii* larvae was determined using the descriptions provided by Uno & Kwon (1969), following their development every alternate day assessed by using an Olympus STM7 dissecting stereo microscope. The larval stage index (LSI) was calculated using the method by Maddox and Manzi (1976): LSI = (∑ Sixni)/N, where *S* is the larval stage, *ni* is the number of larvae in each stage, and N is the total number of larvae examined. Upon conclusion of the experiment, the survival rate (Equation 5), metamorphosis rate (Equation 6), rearing period, and average dry weight of the larvae were

determined. Metamorphosis (recorded by days) was recorded when 95% of the larvae had become post-larvae (PL). Survival and metamorphosis rates were calculated as follows:

For post-larval growth, 50 individuals from each treatment group were sampled and euthanised in iced water. Total length (TL, from rostrum to telson) was measured using digital callipers (± 0.1 cm), and dry weight (DW, mg) was determined by oven-drying at 60°C for 24 hours, then weighing with AT21 Mettler Toledo, Inc. (Shanghai, China; 1 µg).

$$\text{Survival rate (\%)} = (\text{number of post-larvae produced} / \text{number of stocked larvae}) \times 100 \quad [5]$$

$$\text{Metamorphosis rate (\%)} = (\text{final number of post-larvae} / \text{numbers of stocked larvae}) \times 100 \quad [6]$$

Statistical Analysis

The obtained physical properties of the feed, namely flotation rate, water stability, protein, and lipid retention rates, were subjected to a two-way ANOVA. All proportion data were arcsine transformed before analysis. The variation of these features for each feed type over time was analysed using one-way ANOVA. The growth performance data (i.e., survival rate, body length, dry weight and metamorphosis rate, etc.) were evaluated using one-way ANOVA. Where significant differences were found, Duncan's test was used to identify significant differences between the three experimental diets. Statistical analyses were performed using the SPSS version 26.0.

RESULTS AND DISCUSSION

Morphology of Feed Particles

From Figure 1, diet D1 (0% BM & 5% CMC) (Figure 1A) appeared to be more compact. The high content of the binder likely results in a more uniform and cohesive structure with fewer and smaller pores. In contrast to this, diet D2 (3% BM & 2% CMC) (Figure 1B) possessed a rough texture with numerous holes of varying sizes; the binder provided cohesion, the buoyancy modifier helping to create more pores. Lastly, feed D3 (5% BM & 0% CMC) (Figure 1C) displayed a complex porous structure resembling a sponge or coral. The high buoyancy modifier content created more open and interconnected pores, while the absence of the binder resulted in less cohesion, leading to a more open and interconnected structure.

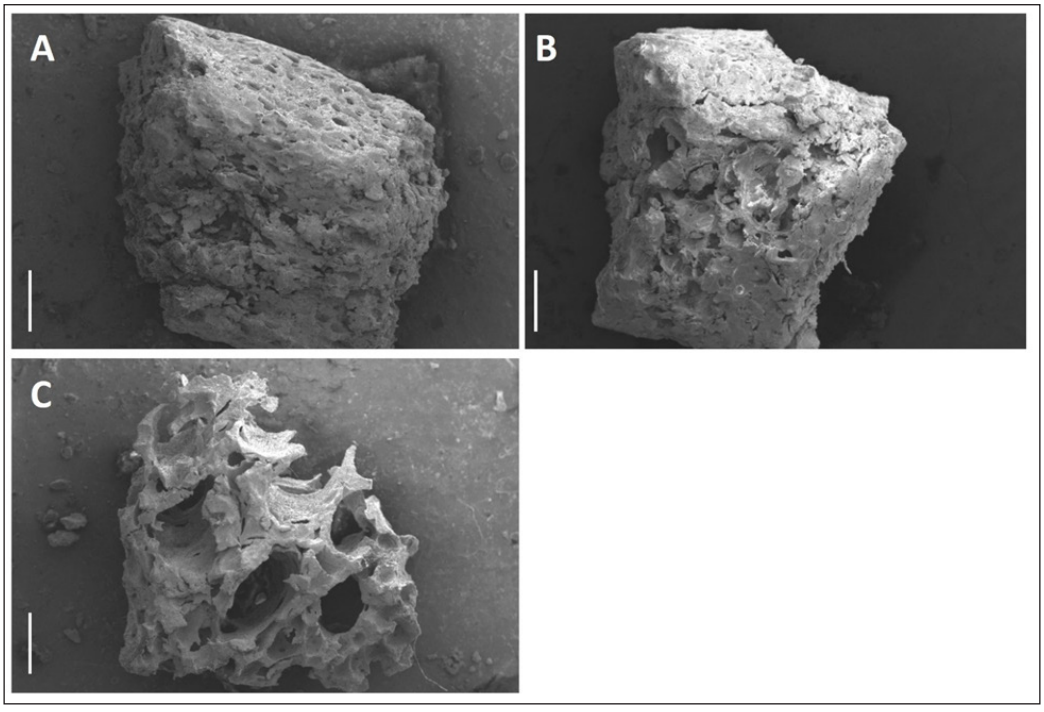

Figure 1. Scanning electron micrographs of representative egg custard particles from each of the three experimental diets. A. Diet D1 with 5% CMC and 0% BM; B. Diet D2 with 2% CMC and 3% BM; C. Diet D3 with 0% CMC and 5% BM. Scale bar = 1 mm

Floatability

The flotation analysis (Table 2) indicated significant effects of both groups ($F(2, 36) = 28.922$, $p < 0.001$) and time ($F(5, 36) = 7.703$, $p < 0.001$) on flotation, with no significant interaction between groups and time ($F(10, 36) = 1.657$, $p = 0.130$). This suggested a consistent effect of time on flotation rates across all diets. D1, D2, and D3 exhibited statistically significant differences in flotation rates ($p<0.05$), following the trend D1 < D2 < D3 (Fig. 2A). The flotation rate gradually declined over time with no significant differences between 10-40 minutes ($p>0.05$), there was, however, a significant decrease from 50 to 60 minutes ($p<0.05$) (Fig. 2B). Proportional flotation showed no significant variations ($p>0.05$) across timepoints, peaking at 20 minutes and declining at 60 minutes for D1. D2 showed a significant decrease by 30 minutes ($p<0.05$) and continued to decline until 60 minutes. D3 remained stable from 10 to 50 minutes, with a significant drop at 60 minutes ($p<0.05$), as shown in Fig. 2C. These results showed that while each experimental diet had different rates of flotation, the general trend over time was similar.

Buoyancy in aquaculture feeds is an important feature that optimises feed accessibility and directly influences the growth and survival of each species (Onomu & Okuthe, 2024).

of the three experimental diets, the control diet (D1, 5% CMC & 0% BM) had the lowest flotation rate after 60 minutes (20.90%, $p<0.05$) (Figure 2A). CMC, a binder, improves pellet integrity and stability, resulting in higher-density particles with a faster sinking rate when used alone at 5% (Attar et al., 2018). The inclusion of 3% BM in D2 and 5% BM in D3 significantly improved the buoyancy of the diets compared to D1, with D3 showing the highest overall flotation rate (Figure 2A, $p<0.05$).

The improved buoyancy of D2 and D3 is linked to the use of yeast (*S. cerevisiae*) as a buoyancy modifier. During fermentation, the yeast produces CO_2, which gets trapped in the feed matrix, creating air-filled spaces that improve buoyancy (Struyf et al., 2017). Similarly, baking powder acts as a chemical leavening agent, releasing CO_2 gas when mixed with moisture and heat, aerating the feed matrix and creating a porous, low-density diet (Gélinas, 2024; Luck, 2021). Together, these ingredients and mechanisms significantly enhance the buoyancy of the diet, allowing it to float effectively in the water column (Orire & Sadiku, 2014). These agents create larger voids within the pellet structure, leading to a porous structure that traps air and results in lighter feed particles (Figure 1B, C).

Flotation rates decreased over time, showing no significant differences between 10 to 50 minutes but a notable decline at 60 minutes (Figure 2B, C). The interaction between dietary groups and time was not significant. The decrease in flotation over time is most likely due to water absorption, and/or feed disintegration

Table 2

The results of a two-way ANOVA assessing three experimental freshwater prawn diets and their interaction on their floatability, water stability, immersion time (measured at 10 min intervals from 10 to 60 min) and capacity for retaining their protein and lipid content. The three diets differed in their inclusion rate of buoyancy modifiers (BM) and carboxymethyl cellulose (CMC, where: D1 (control; 5% CMC; 0% BM); D2 (2% CMC; 3% BM); and D3 (0% CMC; 5% BM). F = F-ratio, and Sig = significance at p≤0.05

Source	Floatability		Stability		Protein Retention		Lipid Retention	
	F	Sig.	F	Sig.	F	Sig.	F	Sig.
Corrected Model	6.643	<0.001	24.818	<0.001	35.275	<0.001	49.138	<0.001
Groups	28.922	<0.001	40.895	<0.001	1.342	0.28	29.459	<0.001
Time	7.703	<0.001	58.719	<0.001	123.572	<0.001	156.636	<0.001
Groups × Time	1.657	0.13	2.509	0.05	2.437	0.06	1.948	0.11

Note. R Squared and Adjusted R Squared:
Floatability: 0.758 (Adjusted R Squared = 0.644)
Stability: 0.919 (Adjusted R Squared = 0.882)
Protein Retention: 0.942 (Adjusted R Squared = 0.915)
Lipid Retention: 0.957 (Adjusted R Squared = 0.938)
Groups and Time Points:
Groups: D1 (5% CMC; 0% BM), D2 (2% CMC; 3% BM), D3 (0% CMC; 5% BM)
Time Points: 10, 20, 30, 40, 50, and 60 minutes

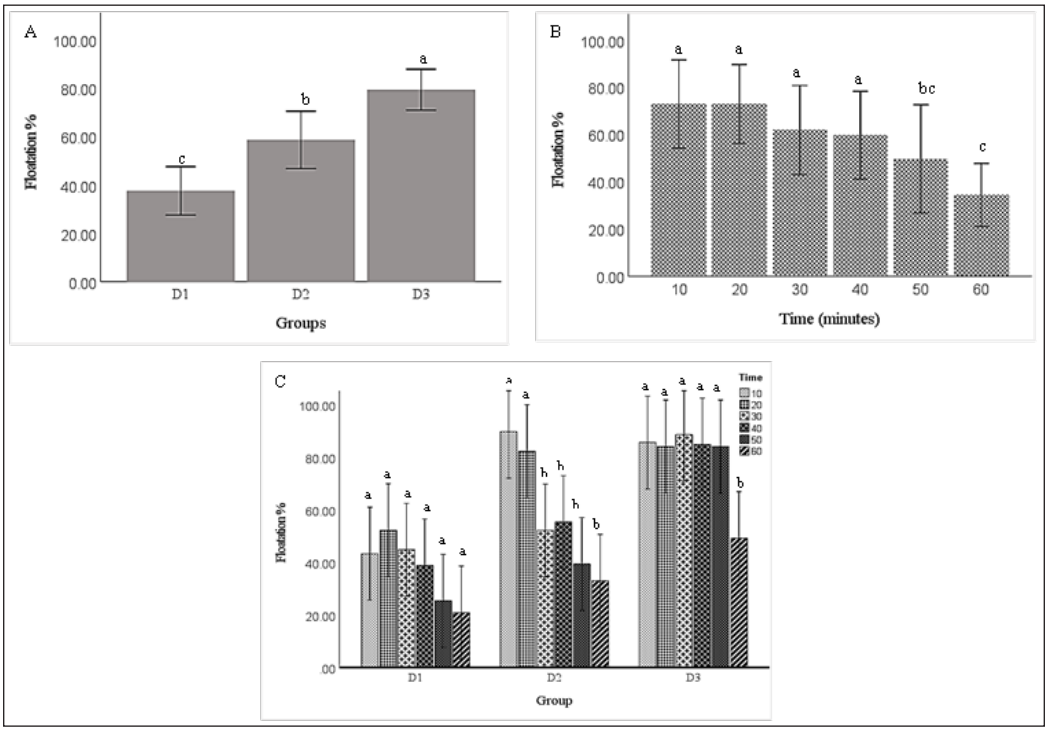

Figure 2. Flotation performance of the three experimental diets (D1-D3); A. Flotation ratios for each diet over a 60-minute period, where D1 = 5% CMC & 0% BM; D2 = 2% CMC & 3% BM, and D3 = 0% CMC & 5% BM); B. The mean flotation ratios averaged across the three experimental diets, without differentiating between diet group or composition, across the 60-minute assessment period; C. Flotation percentages for each diet were determined at 10-minute intervals over the 60-minute assessment. Error bars represent standard deviations of three replicates; different letters indicate statistically significant differences in flotation performance at $p \leq 0.05$

(Chen et al., 2010; October et al., 2019). Lower bulk density, achieved by using lighter ingredients and buoyancy-enhancing formulations, contributes to better floatability in aquafeeds (Ma et al., 2020; Patel et al., 2024; Soares et al., 2021). These studies are consistent with previous studies confirming the use of yeasts and baking powder as buoyancy modifiers, which create larger voids and trap air within feed particles (Adekunle et al., 2014; Falayi & Sadiku, 2013). For example, Adekunle et al. (2014) reported floatation rates of up to 70% using these catalysts, while other binders like cassava starch and Aquatec-11™ achieved similar results (Ma et al., 2021; Orire & Emine, 2019). These results align with the current study, where BM agents are crucial for achieving buoyancy in diets formulated for *M. rosenbergii* larvae. Ensuring 80-100% buoyancy is generally accepted to facilitate efficient feeding behaviour, which is critical for species like *M. rosenbergii*, which are slow feeders (Assan et al., 2021).

Water Stability and Dietary Nutrient Leaching

Table 2 shows that both diet ($F(2, 24) = 40.895$, $p<0.001$) and time ($F(3, 24) = 58.719$, $p<0.001$) significantly affected water stability, with no significant interaction between groups and time ($F(6, 24) = 2.509$, $p = 0.050$). This indicates consistent effects of diet on stability across time intervals. Significant differences were observed between diets over time ($p<0.05$), following the trend D1 > D2 > D3 (Fig. 3A). Water stability progressively decreased over time at each timepoint ($p<0.05$) (Fig. 3B). Within each group, no significant differences were observed from 10 to 40 minutes ($p>0.05$) despite a steady decline. Group D2 showed a similar trend, decreasing to 73.60 ± 0.46 at 10 min and to 69.55 ± 0.35 at 40 minutes. Group D3's stability declined to 72.74 ± 1.27 at 10 min and further to 68.61 ± 0.33 at 40 minutes (Figure 3C).

The findings from Table 2 indicate that time significantly affected protein retention ($F(3, 24) = 123.572$, $p<0.001$), while the type of diet did not ($F(2, 24) = 1.342$, $p = 0.281$). The interaction between group and time was also not significant ($F(6, 24) = 2.437$, $p = 0.055$), implying that the impact of diet on protein retention remained consistent across different time intervals. No significant differences in protein retention were observed among diets D1, D2, and D3 ($p>0.05$), though D1 retained more protein than D2 and D3 (Figure 4A). Protein retention varied significantly over time ($p<0.05$), following a decreasing trend (Figure 4B). Group D1 decreased from 96.40 ± 0.61% at 10 minutes to 89.00 ± 0.80% at 40 minutes (7.40% leaching), group D2 from 97.45 ± 0.49% to 87.60 ± 0.11% (9.85% leaching), and group D3 from 98.01 ± 0.72% to 86.61 ± 0.59% (11.40% leaching) (Figure 4C).

Table 2 also indicates significant effects of both time ($F(3, 24) = 156.636$, $p<0.001$) and diet group ($F(2, 24) = 29.459$, $p<0.001$) on lipid retention percentages. The interaction between diet group and time is not significant ($F(6, 24) = 1.948$, $p = 0.114$), suggesting a consistent impact of diet across different time intervals. Lipid retention significantly differs among diets D1, D2, and D3 ($p<0.05$), following the trend D1 > D2 > D3 (Figure 5A). Lipid retention ratios also significantly vary over time ($p<0.05$) (Fig. 5B), showing a steady decline. The lipid retention by D1 decreased from 97.38 ± 0.41% at 10 minutes to 90.21 ± 1.36% at 40 minutes (7.17% leaching). D2 decreased from 96.42 ± 0.12% to 88.49 ± 1.78% (7.93% leaching), and D3 from 95.82 ± 0.37% to 84.47 ± 0.18% (11.35% leaching) (Figure 5C).

Table 2 highlights the significant differences in water stability results between diets and over time. Higher water stability indicates less dry matter loss, suggesting greater feed cohesion, while lower stability implies greater disintegration or leaching (Lal et al., 2023). Diet D1 (5% CMC & 0% BM) had higher water stability than D2 (2% CMC & 3% BM) and D3 (0% CMC & 5% BM), indicating that dry matter retention decreased as CMC decreased and/or BM increased. D2 exhibited moderate stability when both CMC and

BM were included (Figure 3A). Water stability decreased over time from 10 to 40 minutes (Figure 3B), with no significant interaction between diet and time. Trends in nutrient retention mirrored water stability, with higher retention in D1, followed by D2 and D3 (Figure 4A and 4B). Higher water stability correlated with lower nutrient leaching (Guo et al., 2021), while lower stability led to increased leaching due to faster feed disintegration (Ighwela et al., 2013). CMC enhances feed stability by improving water absorption and forming strong inter-particle bonds, leading to better stability and higher nutrient retention over longer periods (Ayoola, 2020; Fabbrocini et al., 2012). Higher polymerisation and lower substitution rates increase its solubility, contributing to better feed stability. However, BM-based diets showed relatively poor water stability and higher nutrient leaching compared to the control with only CMC addition. This is due to their composition, lacking CMC's strong binding properties in D2 or total binding properties in D3, which, along with increased buoyancy, leads to higher water exposure and nutrient leaching. The nature of yeast and baking powder, components of BM, disrupts CMC's binding properties or dilutes its effectiveness (Abubakar et al., 2016; Solomon et al., 2011). Their higher water absorption enhances buoyancy but challenges water stability, promoting further nutrient loss (Onada & Ogunola, 2019).

CMC produces varied effects on aquafeed performance compared with traditional binders (Adebayo et al., 2003). Studies using CMC reported water stability ranging from 25 minutes to 24 hours (Brown et al., 2015). For example, one study noted 97.53% pellet integrity at 30 minutes, and others documented improvements in pellet durability and water stability for trials lasting up to 3 hours (Aksoy et al., 2022). In contrast to this, other research trials have found that CMC can yield poorer stability relative to corn starch, wheat glucan, or α-starch. For example, in a study conducted by Ayuhafizah et al. (2024) on *M. rosenbergii* larvae and postlarvae, CMC improved pellet stability, but it was less effective than other binders like agar in promoting growth performance. Concentration-dependent effects using CMC have also been reported with optimal water stability and dry matter retention, with an inclusion level of 2-3% CMC. Higher concentrations of up to 14% were associated with poorer feed conversion and reduced lipid content (Brown et al., 2015), suggesting a potential negative impact of excessive CMC on nutrient utilisation. Consistent with this, Yusoff et al. (2021) demonstrated that pellets containing 2% and 3% CMC showed minimal disintegration and good water stability after immersion for 120 minutes. These findings indicate that CMC can enhance aquafeed stability and pellet quality under certain conditions, though its effects on water absorption and nutrient retention are influenced by species, diet formulation, and inclusion rate.

The water stability of the diets ranged from 68.61% -70.80%, the percentage of protein retained ranged from 86.61%-89.01%, and the percentage of lipid retained ranged from 84.47%-90.21%. The greatest loss of dry matter occurred during the first 0-10 minute

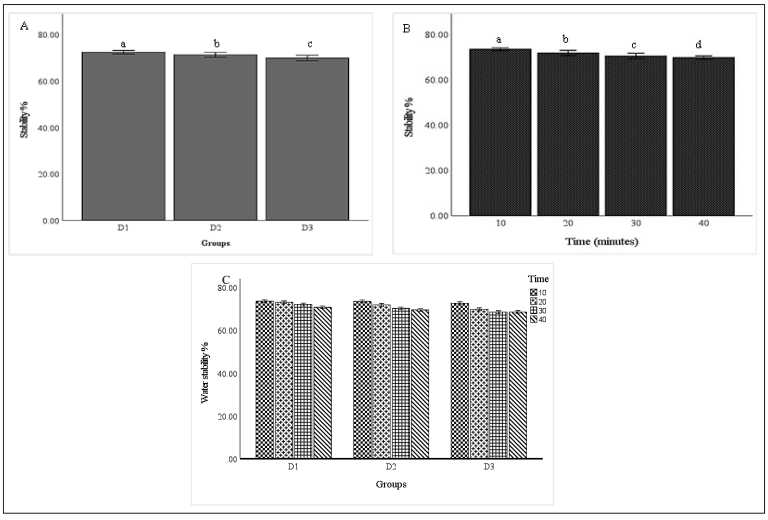

Figure 3. Water stability of the three experimental egg custard diets; A. Mean water stability for each dietary group: D1 = 5% CMC & 0% BM, D2 = 2% CMC & 3% BM, and D3 = 0% CMC & 5% BM, across the 40-minute immersion assessment; B. The mean water stability averaged across all three dietary groups showed the gradual decline in water stability in 10-minute intervals over time the 40-minute assessment period; C. Water stability of each diet following immersion after 10, 20, 30 and 40 minutes. Error bars represent the standard deviation (three replicates). Different superscript letters show significant differences in stability at $p \leq 0.05$

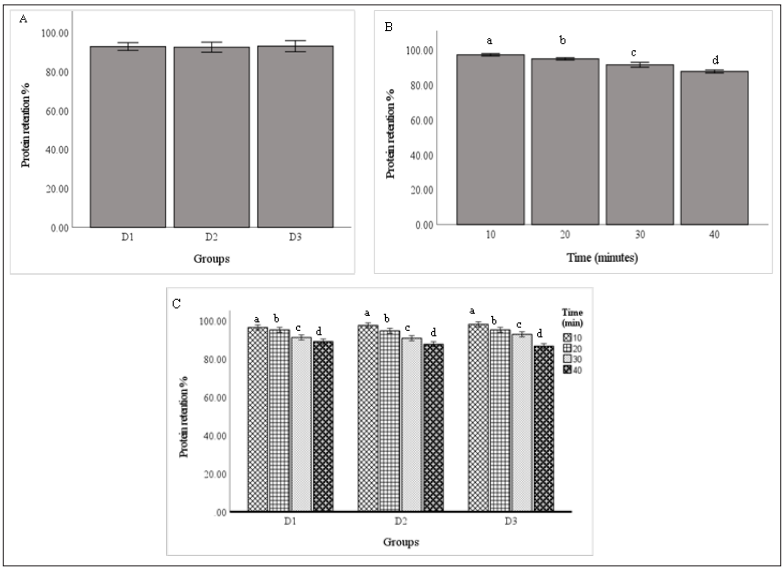

*Figure 4***.** Protein retention rate of the experimental egg custard diets; A. Average protein retention rate for each diet across the entire 40-minute immersion period, where D1 = 5% CMC & 0% BM, D2 = 2% CMC & 3% BM, and D3 = 0% CMC & 5% BM; B. Average protein retention, averaged across all three feed diets, at 10-minute intervals for up to 40 minutes; C. The percentage of protein retained in each diet at each time point (10, 20, 30, and 40 minutes). Error bars represent standard deviations of three replicates; different superscript letters indicate significant differences at $p \leq 0.05$

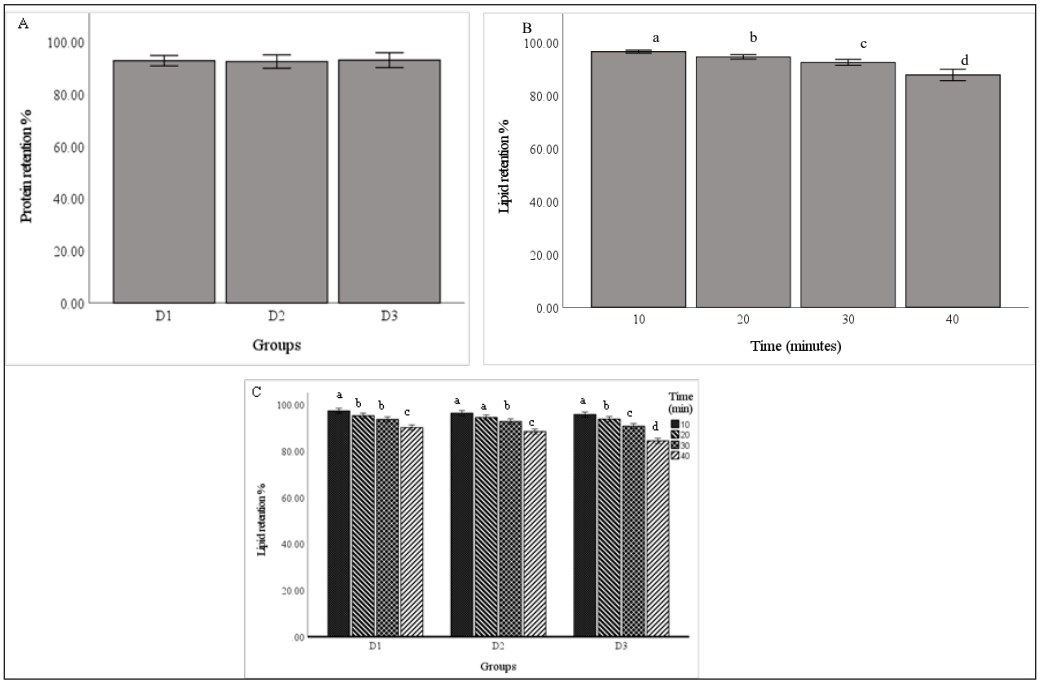

Figure 5. Performance of lipid retention displayed by the different feed groups; A. Differences in lipid retention between the three groups: D1 control: 5% CMC & 0% BM), D2 (2% CMC & 3% BM), and D3 (0% CMC & 5% BM); B. Average lipid retention, averaged across all three feed diets, at 10-minute intervals for up to 40 minutes; C. The percentage of lipid retained by each feed group after immersion in water for 10, 20, 30, and 40 minutes. Error bars represent standard deviations; superscript letters indicate significant differences at $p \leq 0.05$

interval, a period when initial dissolution is most rapid. CMC most likely achieves initial stabilisation by binding ingredients together and forming a protective barrier against water penetration, thus slowing the rate of nutrient leaching. However, prolonged exposure allows water to gradually penetrate the CMC matrix, eventually dissolving and carrying away bound nutrients.

Larval Performance

The study demonstrates the superior performance of diet D2 (2% CMC & 3% BM), followed by diet D3 (0% CMC & 5% BM), compared to the control diet (D1) (Table 3). Diet D2 consistently produced the best results. The control group (D1) exhibited the lowest survival rate at 53.3 ± 3.35%, while the highest survival rates were observed in groups fed diets D2 (66.00 ± 0.54%) and D3 (63.78 ± 2.80%), both of which were significantly higher than the control ($p<0.05$). In terms of dry weight (DW) of PL, diet D2 (1.81 ± 0.10 mg) resulted in significantly higher DW ($p<0.05$) compared to the control group D1 at

1.50 ± 0.10. The DW of larvae fed on D3 (1.66 ± 0.20 mg) showed no statistically significant differences ($p \geq 0.05$) from those fed on D1 and D2. The mean total length (TL) of the PL at the end of the feeding trial ranged from 8.61 to 9.3 mm, with groups fed D2 and D3 showing no significant difference in TL ($p > 0.05$), but both were significantly greater than the control group ($p < 0.05$). Different dietary treatments significantly influenced production and metamorphosis rates ($p < 0.05$). The D2 and D3 treatments had high levels of final mean production (larvae and PL production), which were 39.6 ± 0.33/L and 38.26 ± 1.37/L, respectively, both significantly higher than the control treatment at 31.86 ± 1.64/L ($p < 0.05$). Similarly, the metamorphosis rates in larvae fed D2 and D3 were 62.7 ± 0.51% and 60.58 ± 2.17%, respectively, which did not differ significantly from each other but were considerably higher than those in the control treatment at 50.45 ± 2.60%.

Buoyant feeds play a crucial role in larval crustacean feeding behaviour and nutrient absorption, as feeds that remain suspended in the water column are more accessible to larvae, leading to improved feed consumption, growth, and survival (Langdon & Barrows, 2011). In the present study, the water stability and nutrient leaching outcomes of D2 and D3 were lower than those of D1; however, the *M. rosenbergii* larvae fed on the D2 (3% BM & 2% CMC) and D3 (5% BM & 0% CMC) diets, displayed significantly superior survival and growth performance compared to the control group (5% CMC; 0% BM) ($p < 0.05$). This suggests that incorporating BM into diets effectively maintains feed buoyancy, improving growth outcomes by ensuring larvae can consume sufficient quantities before the feed sinks. The higher BM dosage in D3, without CMC binder, significantly outperformed the control group (D1), though it did not result in further enhancement compared to D2. This suggests that beyond 3% BM, additional performance gains may be limited by nutrient availability rather than buoyancy alone. Therefore, the incorporation of both a binder and a floating agent is a preferable choice.

Table 3
The larviculture performance of Macrobrachium rosenbergii *when fed the three experimental feeds that incorporate different inclusions of CMC and BM*

Performances	5% CMC, 0% BM	2% CMC, 3% BM	0% CMC, 5% BM
	D1	D2	D3
Mean survival (%)	53.11 ± 3.35 ^{b}	66 ± 0.54 ^{a}	63.78 ± 2.8 ^{a}
Body length (mm)	8.61 ± 0.16 ^{b}	9.4 ± 0.20 ^{a}	9.3 ± 0.10 ^{a}
Dry weight (mg)	1.50 ± 0.10 ^{c}	1.81 ± 0.10 ^{a}	1.66 ± 0.20 ^{abc}
Production (larvae + PL/L)	31.86 ± 1.64 ^{b}	39.6 ± 0.33 ^{a}	38.26 ± 1.37 ^{a}
Metamorphosis rate (%)	50.45 ± 2.60 ^{b}	62.7 ± 0.51 ^{a}	60.58 ± 2.17 ^{a}

Note. The values are shown as the mean ± standard deviation resulting from three replicates. Values within the same row with different superscript letters are significantly different ($p < 0.05$). *Abbreviation: BM = buoyancy modifiers; CMC = carboxymethyl cellulose

Several floating agents and binders provide nutrients (Sørensen, 2012). Effective binders contribute to pellet integrity and reduce nutrient leaching, thereby increasing nutrient availability and digestibility (Karim et al., 2022). CMC is a beneficial additive in aquaculture feeds; it acts as a binder, reducing nutrient leaching and disintegration of the pellets, thereby ensuring greater nutrient retention (Ergun et al., 2016; Yusoff et al., 2021). This leads to more efficient utilisation of proteins and dextrins, improved bioavailability, and ultimately, better feed conversion ratios and growth rates (Mohanta, 2024). While effective, CMC works best when combined with other essential feed additives like vitamins and minerals to create a comprehensive approach to sustainable and healthy aquaculture practices (Brum et al., 2025). In a study on spiny lobsters (*Jasus edwardsii*), CMC a highly digestible carbohydrate source was found to enhance nutrient absorption and growth performance in juveniles. Its use in formulated diets, both as a carbohydrate source and as a binder, can significantly improve digestibility and energy utilisation, leading to better growth outcomes (Simon, 2009a, b). It is important to note, however, that these findings are specific to spiny lobsters and may not apply to other species. In contrast to this, a study on largemouth bass (*Micropterus nigricans*) found that dietary CMC negatively impacted growth, nutrient digestibility, and gut morphology, with higher viscosity CMC showing more pronounced adverse effects (Liu et al., 2022). In a study on *M. rosenbergii* larvae and postlarvae, CMC was tested as a binder in microbound diets. The results showed that while CMC improved pellet stability, it was less effective compared to other binders like agar in promoting growth performance. Larvae-fed diets with agar as a binder reached a more advanced developmental stage (Stage XII) when compared to those fed CMC-based diets (Ayuhafizah et al., 2024).

Yeasts and their derivatives, such as brewer's and baker's yeast, offer substantial benefits in aquaculture. Yeasts are rich in proteins, carbohydrates, and bioactive compounds like β-glucans, which can enhance growth, survival, and feed efficiency across various species (Álvarez-Sánchez et al., 2018; Chotikachinda et al., 2008). Several studies have demonstrated that yeast supplements can effectively replace fishmeal and can represent a cost-efficient alternative without compromising growth performance (Huang et al., 2024; Pongpet et al., 2016). Additionally, the probiotic and immunostimulant properties of yeasts can help improve disease resistance and nutrient utilisation (Cai et al., 2023; Nhi et al., 2018; Zhang et al., 2018). Combining CMC and BM into a larval diet for *M. rosenbergii* aligns with their behavioural preferences for readily accessible and nutritionally stable food sources, leading to superior larval performance. The cost of diet production is another important consideration; while both CMC and BM are relatively inexpensive ingredients, further research is needed to determine the practical limits of BM inclusion and its potential impacts on palatability, water quality, nutrient density, and overall cost-effectiveness in relation to larval performance.

CONCLUSION

This study highlights that while the inclusion of CMC improves water stability and reduces nutrient leaching, it alone does not ensure optimal buoyancy for *M. rosenbergii* larvae. The control diet with 5% CMC had the best water stability and nutrient retention; however, this resulted in poor growth. Adding BM to the feed reduced nutrient retention and water stability but helped enhance buoyancy. By comparison, the diets with 3% BM and 2% CMC (D2) and 5% BM and 0% CMC (D3) showed superior growth and survival. From the experimental dietary formulations considered here, using 3% BM with 2% CMC resulted in maximal growth and survival of the *M. rosenbergii* larvae. The study concludes that customising the inclusions of binder and buoyancy modifier according to specific needs can help enhance larval performance.

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CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

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Andrew P Shinn	:	Supervision, writing - review and editing.
Rasina Rasid	:	Conceptualisation, supervision, project administration, funding acquisition, formal analysis, writing - review and editing.

DATA AVAILABILITY

All data presented within this article is available on request.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest or personal relationships that could have appeared to influence the work reported in this paper.

DECLARATION OF GENERATIVE AI USE

During the final revision of this manuscript, the authors used Claude.ai. to improve the text for clarity and to correct any grammatical errors. Following use of the tool, the authors take full responsibility for the content of the published article.

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