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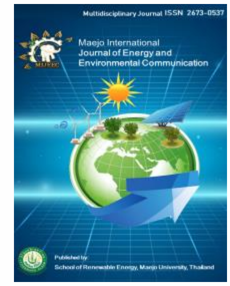

ARTICLE

Evaluation of carbohydrate sources in formulated feeds on growth performance, survival, and environmental sustainability of juvenile spotted babylon (*Babylonia areolata*) in a flow-through seawater system

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ABSTRACT

Thailand is investigating the potential of utilizing spotted babylon (*B. areolata*) as a substitute protein source to tackle discarded fish feed's expensive and unreliable nature. This study assesses the cost-effectiveness and nutritional balance of diets over a period of 180 days. The diets include various carbohydrate sources, each making up 3% of the total diet. These sources are *Gracilaria fisheri*, *Ulva reticulata*, *Ulva intestinalis*, and *Metroxylon sagu*. 1,200 juveniles were fed the meals under controlled conditions to observe their growth, feed conversion ratios (FCR), and survival rates. The findings indicated that diets containing *U. reticulata* led to superior growth performance, with a feed conversion ratio (FCR) of 0.71±0.07, which was considerably better than the FCR of 1.48±0.07 observed in the control diet. *G. fisher*i demonstrated excellent performance, with a feed conversion ratio (FCR) of 0.93±0.03. Diets containing *M. sagu* and *U. intestinalis* exhibited lower efficiency. The diets were found to be highly suitable for juvenile spotted babylon, as shown by the high survival rates above 95% in all groups. The results emphasize the potential of using alternative carbohydrate sources like as *U. reticulata* and *G. fisheri* to improve growth and feed efficiency in aquaculture.

1. Introduction

Aquaculture is rapidly expanding, primarily in lower- and middle-income countries, outpacing global population growth (Chaimongkol et al., 2023; Klahan et al., 2023). This growth presents opportunities to enhance production efficiency, modernize systems, and boost exports, improving economic and social development (Yongphet et al., 2016). Sustainable growth requires dynamic governance involving equity, accountability, efficiency, and predictability (Pimpimol et al., 2020; Tongmee et al., 2021a). Over the past decade, countries have revised governance and enacted regulations to increase productivity across the aquaculture value chain (Bhuyar et al., 2021; Tongmee et al., 2021b). Producers have adhered to international and domestic regulations, leading to industry restructuring (Whangchai et al., 2018). Thailand's aquaculture sector is crucial for food security and the economy, with freshwater aquaculture used for domestic consumption and brackish

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water aquaculture producing high-value export products (Yongphet et al., 2020, Whangchai et al., 2022a).

Aquaculture of the marine gastropod spotted babylon (*Babylonia areolata*) is beginning to show promise as a novel source of protein for human consumption. There is a growing worldwide need for seafood, particularly spotted babylon, which is highly valued in the market (Sutthinon et al., 2023). This has led to Thailand's strong interest in developing aquaculture methods for this species due to concerns about the sustainability of wild stocks (Chaitanawisuti et al., 2002; Dobson et al., 2021).

Historically, this mollusk has been cultivated in Thailand by utilizing chopped waste fish as the main source of nutrition during the phase of growth and development. Nevertheless, this approach poses several obstacles: the feed is expensive, its availability could be more stable, its nutritional value might be uncertain, it is challenging to store, and it frequently deteriorates water quality, perhaps causing the transmission of diseases. The scarcity of garbage fish throughout the 6-7 month cultivation period presents a significant obstacle to the commercial production of this species. Therefore, it is crucial to design a feed formulation for spotted babylon aquaculture that is both cost-effective and nutritionally balanced in order to ensure future success (Chaitanawisuti & Kritsanapuntu, 1999; Zhou et al., 2007a,b).

In order to develop a prosperous aquaculture sector for spotted babylon, it is essential to enhance their nutrition in order to achieve accelerated growth, elevated survival rates, and superior flesh quality. An obstacle of great importance in attaining large-scale aquaculture is creating an efficient and feasible diet (Whangchai et al., 2022). Although numerous studies have investigated different foods' effects on Spotted Babylon's growth, these diets typically fail to reproduce the growth rates observed with natural diets. Creating an effective feed that improves growth performance is crucial in building a sustainable aquaculture sector for spotted babylon in Thailand. Additional investigation into this predatory aquatic species is vital to enhance growth rates, optimize feed efficiency, and reduce waste outputs, guaranteeing spotted babylon cultivation's economic and developmental viability (Xue et al., 2010).

Protein is a macronutrient that is given considerable importance in nutritional studies because it is expensive in commercial feeds and plays a crucial role in the growth of fish and shellfish, as emphasized by Zhou et al. (2007a). Zhou et al. (2007b) further highlighted that aquaculture species can fulfill their energy needs by utilizing a mix of protein, fat, and carbohydrate sources. Dietary lipids are critical for carnivorous snails as they supply concentrated energy, necessary fatty acids, phospholipids, sterols, and fat-soluble vitamins. This is especially important because these snails may be restricted to using carbohydrates as an energy source.

Multiple studies have shown that maintaining sufficient dietary lipids can decrease the need for costly protein as an energy source (Lopez et al., 2006; Li et al., 2023). Hence, attaining an ideal equilibrium of protein, carbohydrate, and lipid proportions in the diet is crucial to stimulating growth performance, improving product quality, and developing efficient snail meals. It is similarly crucial to identify appropriate sources of carbohydrates for this species. To enhance production processes for spotted babylon, it is imperative to decrease the expenses associated with dietary materials and composition. This will enable the development of more cost-effective diets that maintain excellent growth performance and feed efficiency.

This study addresses the current obstacles in spotted babylon aquaculture by examining how different dietary carbohydrate sources impact the growth performance of young spotted babylon (*Babylonia areolata*). The project aims to find economically efficient and nutritionally well-balanced feed compositions that can substitute the conventional rubbish fish diet. This will help solve concerns about availability, cost, and the adverse effects on water quality. This study aims to analyze various carbohydrate sources over a 180-day experimental period to gain valuable insights into dietary formulations that enhance growth, survival, and flesh quality. Additionally, it aims to contribute to the sustainability and economic feasibility of spotted babylon aquaculture in Thailand.

2. Materials and methods

2.1 Experimental design and diets

This study aimed to assess the influence of various food carbohydrate sources on the growth performance of juvenile spotted babylon (*B. areolata*) during a 180-day duration.

Table 1. Composition of experimental formulated feeds (g/100g diet)

Ingredients	Treatment				
	1	2	3	4	5
Yellow Stripe Trevally Fish	100	-	-	-	-
Fishmeal	-	40	40	40	40
Soybean meal	-	5	5	5	5
Wheat flour	-	21	21	21	21
Shrimp head meal	-	5	5	5	5
Wheat gluten	-	15	15	15	15
Glacilaria fisheri	-	3	0	0	0
Ulva reticulata	-	0	3	0	0
Ulva intestinalis	-	0	0	3	0
Metroxylon sagu	-	0	0	0	3
Tuna oil	-	5	5	5	5
Vitamins mix[1]	-	2	2	2	2
Minerals mix[2]	-	2	2	2	2
Calcium hydrogen phosphate	-	2	2	2	2
Total	100	100	100	100	100

[1]Vitamin mix (g/diet 1 kg): Retinal(A) 150,000,000 IU; Cholecalciferol(D3) 3,000,000 IU; Tocopherol(E) 27.5; Menadione sodium bisulfate(K3) 4.67; Thiamine(B1) 25; Calcium pantothenate 5; Folic acid 0.4; Ascorbic acid 143

[2]Mineral mix (g/diet 1 kg): Ca 11.22; Mg 2.48; Cu 1.25; Zn 2.14; Na 106.22; squid liver powder 100

Four experimental treatments, each with three duplicates, were carried out utilizing designed meals with four distinct carbohydrate sources, each making up 3% of the total contents. The carbohydrate sources utilized included *Gracilaria fisheri* (GF), *Ulva reticulata* (UR), *Ulva intestinalis* (UI), and Metroxylon sagu (MS). The control diet (FM) consisted of fresh meat from yellow stripe trevally (*Selaroides leptolepis*). The elements and their near composition values are specified in Table 1. The main source of protein in the meal was fish, whereas the source of lipids was tuna oil. The dry components were separately measured and meticulously blended using an electric grinder. Before incorporating the other ingredients, vitamin and mineral premixes were individually blended with cellulose. Subsequently, oils were incorporated into the dry mixture and well-blended once more.

Water was introduced into the mixture, and the resultant damp combination was manually manipulated until it transformed into a rigid dough. Next, the dough was transformed into pellets by passing it through a hand mincer equipped with a 3 mm die. The pellets were stored in durable plastic bags with a high gauge and preserved in a refrigerator. The feed was subjected to proximate analysis using established procedures (AOAC, 1990). Each experimental feed was randomly assigned to four tanks, and the feeding trial lasted for 180 days.

2.2 Husbandry conditions

2.2.1 Acclimatization

The young spotted babylon (*B. areolata*) utilized in the feeding trials were obtained from Aquatic Animal Hatchery and Research. The specimens were transferred to the wet laboratory and housed in two 2,000-liter cylindrical plastic tanks for acclimatization. Throughout this 10-day duration, the snails were provided with diced refuse fish as their diet until they displayed no indications of stress or debilitation.

2.2.2 Initial sorting

At the commencement of the experiment, healthy young snails were meticulously arranged according to a consistent size in order to avoid any possible stunting of growth in smaller snails when they were cultured alongside larger ones. The young individuals were measured with an electronic balance, with a precision of 0.01 g. A total of 1,200 juveniles, all with the same body weight of 0.5 g, were divided into 18 plastic raising tanks. Each tank had dimensions of 0.80 x 0.55 x 0.50 m and contained 80 snails, resulting in a density of 180 snails per square meter.

2.2.3Feeding procedure

During the feeding experiments, young individuals were manually fed once a day at 10:00 AM until they reached a point of visual satisfaction with the experimental food. The daily feed allocation was adjusted depending on the snails' consumption within a 0.5-hour period the previous day, aiming to reduce feed wastage. Satiation was assessed by monitoring where the snails stopped actively consuming food, and relocated from the feeding area,. Every day, uneaten food was removed from the water after the snails ceased eating to avoid the deterioration of water quality. The daily feed consumption was recorded to compute the feed conversion ratio.

2.2.4 Water and tank management

Each rearing tank had a continuous flow water system, maintaining a consistent flow rate of 16 liters per minute for 12 hours daily. A substrate of coarse sand, with a thickness of 1.0 cm, covered the bottom of the tank. The water level in the tanks was consistently maintained at a depth of 30 cm. The tanks were fitted with additional aerator stones, and the water underwent aeration for a duration of 20 hours each day. A light cycle of 12 hours and 12 minutes was maintained. The water temperature was maintained at a constant level of 29±2°C, while the salinity was maintained at 30 parts per thousand (ppt) by adding well water. The water quality parameters were assessed, and the following values were recorded: dissolved oxygen levels over 6.50 mg/l, total ammonia nitrogen levels below 0.30 mg/l, nitrite levels below 0.45 mg/l, and pH levels above 7.8. Weekly maintenance of the aquaria involved siphoning out 75% of the water to eliminate surplus feed and fecal waste and scrubbing the tank walls. The experiment was conducted without the use of any drugs or antibiotics, and there was no sorting of the organisms based on size during the growth period.

2.2.4 Sampling and measurements

Sampling Procedure: At regular intervals of 30 days, a random selection of 20 snails was taken from each treatment group. The weight and length of each snail were measured individually with a precision of 0.01 g. Each tank was monitored daily to record the number of deceased snails, and the survival rate was determined at the conclusion of the experiment.

The following equations were used to assess growth performance:

- Weight Gain (WG): WG = Final body weight (g) – Initial body weight (g)
- Specific Growth Rate (SGR): SGR (% day^{-1}) = 100 x [ln (final weight, g) – ln (initial weight, g) / (culture period, days)]
- Survival Rate (%): Survival (%) = (Final number of snails – Initial number of snails) x 100 / Initial number of snails
- Food Conversion Ratio (FCR): FCR = (dry weight of feed consumed, g) / (wet weight gain of snail, g)

2.3 Statistical analysis

Statistically analyzed data were analyzed by variance analysis (ANOVA) using the SPSS program, version 11.5. The effect of different dietary carbohydrate sources on FBW, WG, SGR, and FCR data was subjected to a one-way ANOVA. Duncan's multiple range test compared differences between treatment means when significant F values were observed at $P<0.05$.

3. Results

3.1 Nutrition values of experimental feeds

A proximate analysis of the four experimental feeds containing different carbohydrate sources was presented in Table 2. Results showed that the crude protein of the control feed (82.43%) was significantly higher than those of the four experimental feeds containing the dietary carbohydrate sources from *Glacilaria fisheri* (40.39%), *Ulva reticulata* (40.88%), *Ulva intestinalis* (41.17%) and *Metroxylon sagu* (40.82%), respectively, while, the ash level of the control feed (4.43%) was significant lower than those of all experimental feeds containing the dietary carbohydrate sources from *G. fisheri* (16.45%), *U. reticulata* (16.63%), *U. intestinalis* (17.59%)

and Metroxylon sagu (16.14%), respectively.

3.2 Growth performance

Growth performance in total body weight and total length of juvenile *B. areolata* fed diets containing different carbohydrate sources for 180 days was presented in Tables 3 and 4. Results showed that the average weight gain (AWG), weight gain (WG), length gain (LG), and absolute growth rate (AGR) were significantly affected (P<0.05) by the experimental feeds containing different dietary carbohydrate sources (Table 5). Juveniles fed with control feed had the highest absolute growth rate in weight (1.02+0.06 g/mo-1), followed by those fed with *U. reticulata* (0.71+0.07 g/mo-1) and *G. fisheri* (0.67+0.10 g/mo-1), respectively. At the same time, the lowest one was found in juveniles fed with Metroxylon sagu (0.60+0.06 g/mo-1), *U. intestinalis* (0.39+0.08 g/mo-1), respectively (Table 5).

Table 2. Proximate analysis of raw material in formulated diet using different carbohydrate sources for spotted babylon *Babylonia areolata* feeds (% dry weight basis)

Treatment	Protein (%)	Lipid (%)	Moisture (%)	Ash (%)
T1(control)	82.43+0.50	8.90+0.20	5.86+0.45	4.43+0.19
T2 *Glacilaria fisheri* 3% (GF3)	40.39±0.09	8.12±0.18	8.42±0.33	16.45±0.11
T3 *Ulva reticulara* 3% (UR3)	40.88±0.20	9.54±0.14	8.06±0.19	16.63±0.18
T4 *Ulva intestinalis* 3% (UI3)	41.17±0.21	11.40±1.19	7.65±0.06	17.59±0.17
T5 *Metroxylon sagu* 3% (MS3)	40.82+0.06	11.11+0.37	7.72+0.45	16.14+0.26

Table 3. Average total weight of juvenile spotted babylon snails fed with formulated diets using different carbohydrate sources during 6fors experiment

	Weight (g)						
Treatment	initial	1 month	2 months	3 months	4 months	5 months	6 months
T1(control)	0.52+0.05[a]	1.29+0.15[b]	2.30+0.23[b]	3.35+0.38[c]	4.20+0.39[c]	5.16+0.31[c]	6.65+0.36[c]
T2(GF3)	0.49+0.04[a]	0.97+0.09[a]	1.66+0.19[a]	2.42+0.20[b]	2.82+0.24[b]	3.64+0.55[b]	4.53+0.58[b]
T3(UR3)	0.50+0.03[a]	1.02+0.05[a]	1.72+0.18[a]	2.55+0.18[b]	2.97+0.25[b]	3.67+0.24[b]	4.78+0.40[b]
T4(UI3)	0.51+0.04[a]	0.97+0.11[a]	1.39+0.36[a]	1.61+0.39[a]	1.84+0.05[a]	2.31+0.60[a]	2.88+0.53[a]
T5(MS3)	0.49+0.04[a]	1.02+0.07[a]	1.75+0.21[a]	2.42+0.19[b]	2.65+0.46[b]	3.29+0.14[b]	4.07+0.38[b]

Note: Data represented as Mean±SD. Value in the same column with different superscripts are significantly different (p<0.05)

Table 4. Average shell length of juvenile spotted babylon snails fed with formulated diets using different carbohydrate sources during 6fors experiment

	Length (cm)						
Treatment	initial	1 month	2 months	3 months	4 months	5 months	6 months
T1(control)	1.36+0.04[a]	1.78+0.08[b]	2.18+0.08[b]	2.47+0.06[c]	2.68+0.05[c]	2.78+0.08[c]	3.09+0.04[d]
T2(GF3)	1.35+0.05[a]	1.62+0.05[a]	1.91+0.06[ab]	2.17+0.08[b]	2.32+0.05[b]	2.47+0.14[b]	2.66+0.08[bc]
T3(UR3)	1.36+0.04[a]	1.66+0.04[a]	1.96+0.07[b]	2.22+0.04[b]	2.35+0.07[b]	2.50+0.06[b]	2.76+0.07[c]
T4(UI3)	1.36+0.02[a]	1.62+0.09[a]	1.80+0.16[a]	1.92+0.16[a]	2.01+0.20[a]	2.16+0.20[a]	2.37+0.08[a]
T5(MS3)	1.33+0.05[a]	1.65+0.06[a]	1.95+0.11[ab]	2.21+0.11[b]	2.24+0.15[b]	2.40+0.08[b]	2.61+0.10[b]

Note: Data represented as Mean±SD. Value in the same column with different superscripts are significantly different (p<0.05)

Table 5. Average shell width of juvenile spotted babylon snails fed with formulated diets using different carbohydrate sources during 6 months experiment

	Width (cm)						
Treatment	initial	1 month	2 months	3 months	4 months	5 months	6 months
T1(control)	0.86+0.05[a]	1.26+0.05[b]	1.52+0.05[b]	1.73+0.06[c]	1.83+0.05[c]	1.94+0.04[c]	2.13+0.03[d]
T2(GF3)	0.86+0.04[a]	1.17+0.03[a]	1.36+0.05[a]	1.54+0.05[b]	1.60+0.05[b]	1.71+0.10[b]	1.83+0.07[bc]
T3(UR3)	0.85+0.03[a]	1.19+0.02[a]	1.37+0.03[b]	1.58+0.03[b]	1.62+0.05[b]	1.75+0.05[b]	1.89+0.05[c]
T4(UI3)	0.87+0.02[a]	1.17+0.04[a]	1.28+0.10[a]	1.35+0.10[a]	1.39+0.11[a]	1.51+0.13[a]	1.64+0.08[a]
T5(MS3)	0.84+0.02[a]	1.18+0.03[a]	1.37+0.06[a]	1.54+0.05[b]	1.55+0.09[b]	1.65+0.02[b]	1.79+0.04[b]

Note: Data represented as Mean±SD. Value in the same column with different superscripts are significantly different (p<0.05)

3.3 Food conversion ratio (FCR)

Significantly, differences in food conversion ratio (FCR) were found among snails fed with the control feed and the four experimental feeds containing different dietary carbohydrate sources ($P<0.05$). Results showed that juveniles fed with control feed had the highest FCR (1.48+0.07), followed by those fed with *M. sagu* (1.03+0.11) and *U. intestinalis* (0.93+0.03), which was significantly higher than FCR of those fed with *U. reticulata* (0.71+0.07) and *G. fisheri* (Table 5).

4. Discussion

The present investigation showcases the largest and statistically significant enhancement in development and feed efficiency for young *B. areolata* when provided with a diet containing a protein-to-energy (P:E) ratio of 88.66 mg protein/kcal. Young individuals with a diet containing 36% protein and 10% lipid showed the most significant increase in growth and efficiency in converting food into body mass (Zhou et al. 2007a, b). Augmenting the protein content within these lipid levels led to up to 36% growth rate improvements. The survival rates remained consistently high, surpassing 95% in all of the feeding studies (Chen et al., 2015). Therefore, the study suggests a diet consisting of 36% protein and 10% fat while maintaining a P:E ratio of 88.66 mg protein/kcal.

This diet is recommended for achieving optimal development and feed efficiency in juvenile *B. areolata*. This recommendation contradicts the previous research conducted by Zhou et al. (2007a, 2007b). Zhou et al. found that juvenile *B. areolata* requires higher protein intake for optimal growth, specifically 45% protein. They also observed that weight gain was negatively affected by lipid levels above 7.80%, and the optimal lipid requirement was around 6.54%.

In their study, Lee & Kim (2005) emphasized the distinct nutritional requirements for snails (*Semisulcospira gottschei*). They suggested a meal consisting of 22% protein, which should have a reduced calorie content and a P ratio of 69 mg protein/kcal. In addition, they emphasized the significance of n-3 unsaturated fatty acids and proposed plant oils as suitable energy sources after the requirements for these fatty acids are fulfilled (Xue et al., 2010).

The study's results highlight a correlation that is comparable to what has been observed in other gastropods, such abalone, where an increase in protein content has a favorable impact on growth (Britz, 1996; Mai et al., 1995a,b). However, differences in nutritional preferences among species, like as the carnivorous spotted babylon and the omnivorous or herbivorous abalone, may explain disparities in the efficacy of their diets (Li et al., 2023).

Moreover, the research revealed that diets containing 36% protein and smaller dietary fat (10%) resulted in the most significant growth indices and protein utilization efficiency. These findings support the importance of essential fatty acids, as highlighted by Zhou et al. (2007b), with protein being identified as the main nutrient for growth. The findings suggest that diets with P ratios ranging from 85.36 to 88.66 mg protein/kcal yielded similar growth and feed consumption rates, surpassing diets with lower P:E ratios.

Hewitt (1992) discovered that an imbalance in the dietary protein-to-energy (CP) ratio might cause an increase in protein breakdown and decrease protein retention in crustaceans. Therefore, the study highlights the significance of the dietary P ratio rather than only the protein or calorie intake in the diet.

Ultimately, additional investigation is required to examine the effects of different dietary proteins, lipids, and carbohydrates on the growth of juvenile *B. areolata* to ensure that growth is not hindered. Modifying the proportions of carbohydrates and lipids in the diet could potentially improve growth outcomes. However, it is important to ensure that critical fatty acid requirements are met and to evaluate the feasibility of producing such a diet on a wide scale.

5. Conclusion

This study confirms that using alternative carbohydrate sources in the diets of juvenile spotted babylon (*B. areolata*) can improve growth performance and feed efficiency. This represents a significant departure from the traditional reliance on waste fish, which has been problematic. The inclusion of *U. reticulata* and *G. fisheri* in the diets resulted in the best growth outcomes, with significantly lower feed FCRs compared to the control diet. This indicates both economic and nutritional advantages. These findings not only support the potential for these carbohydrate sources to replace conventional feed ingredients, but also highlight the broader implications for sustainable aquaculture practices in Thailand. The high survival rates and improved feed efficiency observed in the experimental groups further validate the effectiveness of these diets. Moving forward, it is important to refine these diet formulations.

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