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Full Length Research Paper

Optimum dietary protein levels and protein to energy ratios on growth and survival of juveniles spotted Babylon (*Babylonia areolata* Link) under the recirculating seawater conditions

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A feeding experiment of three dietary protein levels (35, 40 and 45%) and three dietary energy levels (3.8, 4.0 and 4.2 kcal g^{-1} diet) factorial design with four replicates was conducted to determine the proper dietary protein and energy levels for the growth and survival of the juveniles spotted Babylon *Babylonia areolata* under the recirculating seawater conditions. Snails with initial body weight averaging 0.29 ± 0.01 g were fed the experimental diets for 16 weeks. Survival rates (SR) of each group was above 95% and no significant difference among dietary treatments ($p > 0.05$). Mean weight gain of snails fed the 35% protein diets with 4.0 kcal/g diet was significantly ($P < 0.05$) different from that of snails fed the 40 and 45% protein diets at all energy levels. Feed conversion ratio (FCR) was significantly influenced by dietary protein and energy levels ($p < 0.05$). Diets containing 35% protein level and 4.0 kcal g^{-1} energy level appeared to be utilized more efficiently in term of FCR (3.21) than diets containing the other dietary protein and energy levels, ranging 3.62 - 4.54. The results of this study indicate that a diet containing 35% dietary protein and 4.0 kcal g^{-1} diets with P/E ratio of 85.99 mg protein/kcal was recommended for juvenile *B. areolata* growth under our experimental recirculating seawater conditions.

Key words: *B. areolata*, dietary protein, protein / energy ratio, growth, survival.

INTRODUCTION

The spotted Babylon, *Babylonia areolata*, is generally carnivores and feed mostly on fresh meat of trash fish. However, feeding fish meat to spotted Babylon entails problems such as variability in nutritive content and supply, thus resulting a slow and heterogenous growth rate of the species. Typical growth rates of spotted Babylon are approximately 1.19 g mo^{-1} and therefore, 6 - 7 months are required to produce a marketable size. Due to problems associated with the use of trash fish as feed for spotted babylon, intensive spotted Babylon culture is becoming increasingly reliant upon formulated practical diets (Chaitanawisuti Kritsanapuntu and Natsukari, 2002). The use of prepared feeds can be very practical since formulation can be manipulated to obtain an optimum nutritional value. Further, they are available on demand and if properly prepared may be stored for a long time. The use of formulated feeds in spotted Babylon farming will therefore make a significant contribution to spotted Babylon production in Thailand. Zhou et al. (2007) found that the optimal dietary protein requirement for maximum growth rate and feed of *Babylonia areolata* increased with an increase in protein content from 27 - 45%. The success of intensive fish culture depends to a large extent on adequate information on nutrient requirements,

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Table 1. Percentage (dry weight basis) and proximate analysis (%) of the formulated diets and natural food.

Ingredients (%)	Protein levels (%)								
	35			40			45		
	Gross energy level (kcal g^{-1})								
	3.8	4.0	4.3	3.8	4.0	4.3	3.8	4.0	4.3
Fishmeal	45.12	45.12	45.12	50.76	50.76	50.76	50.76	50.76	50.76
Soybean meal	9.48	9.48	9.48	8.06	8.06	8.06	11.85	11.85	11.85
Shrimp meal	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4
Wheat flour	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Wheat gluten	4.77	4.77	4.77	4.77	4.77	4.77	4.77	4.77	4.77
Tuna oil	6.54	11.54	14.54	6.17	11.17	14.17	6.1	11.1	14.1
Vitamin premix[a]	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Mineral premix[b]	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Cellulose	20.69	15.69	12.69	16.84	11.84	8.84	13.12	8.12	5.12
Proximate composition									
Crude protein (%)	35.56	35.21	35.24	40.11	40.63	40.87	45.32	45.25	45.67
Crude lipid (%)	9.67	14.99	18.25	10.11	14.52	18.02	10.04	15.09	18.02
Ash (%)	20.86	20.70	20.78	20.56	20.67	20.84	20.44	20.73	20.55
Energy (kcal /g diet)	3.89	4.07	4.27	3.83	4.05	4.19	3.72	3.93	4.16
P/E ratio (mg/kcal)	89.97	85.99	81.96	104.43	98.76	95.46	120.96	114.50	108.17

[a] Vitamin premix (mg kg^{-1} or IU): vitamin A, 10000000 IU; vitamin D3, 1000000 IU; vitamin E, 10000 mg kg^{-1}; vitamin K3, 1000 mg kg^{-1}; vitamin B1, 500 mg kg^{-1}; vitamin B2, 5000 mg kg^{-1}; vitamin B6, 1500 mg kg^{-1}; vitamin C, 10000 mg kg^{-1}; folate, 1000 mg kg^{-1}; dealmethionine, 16038 mg kg^{-1}.

[b] Mineral premix (mg kg^{-1}): Ca, 147 g kg^{-1}; P, 147 g kg^{-1}; Fe, 2010 mg kg^{-1}; Cu, 3621 mg kg^{-1}; Zn, 6424 mg kg^{-1}; Mn, 10062 mg kg^{-1}; Co, 105 mg kg^{-1}; I, 1000 mg kg^{-1}; Se, 60 mg kg^{-1}.

especially dietary protein, which is the most essential but expensive component in artificial diets. A dietary excess or deficiency of energy can reduce growth rate of farmed fish because energy is needed for metabolic activities (Sweilum Abdella and Eldin, 2005). The use of protein as a dietary energy source for aquatic animals is undesirable because of the high cost of dietary protein as compared with the cost of non-protein energy sources. Increasing protein level in diets can lead to improve production, especially for carnivorous species. However, excessive dietary protein level is not economical for fish and shellfish culture because it is responsible for a large part of the feed cost (Lee and Kim, 2001). However, protein and energy levels are known to influence the growth and body composition of various fish and shellfish. With respect to dietary energy levels, Shiau and Huang (1990) found that FCR and protein efficiency ratio of hybrid tilapia *Oreochromis aureus* were improved at dietary energy levels higher than 12.97 kj 100 g^{-1} diet when the fish fed a diet containing 25% protein. Similarly, El-Sayed and Teshima (1992) reported that at 30% dietary protein, fish growth and feed utilization were significantly improved by increasing the dietary energy level from 12.56 - 20.93 kj 100 g^{-1} diet. In addition, a proper balance of crude protein and digestible energy can improve growth rate, feed efficiency and protein utilization; minimizes excessive accumulation of lipids and glycogen in the somatic tissues and liver; and minimizes undesirable nitrogenous waste output thereby improving the quality of farm effluents (Bicuda Sado and Cyrino, 2009). It is important to know the response of spotted Babylon to various nutrients in order to be able to produce an effective low-cost feeds for the species. Hence, this study was designed to determine the effects of different levels of dietary protein and energy in formulated diets on growth of juveniles spotted Babylon *B. areolata*, reared under the recirculating system.

MATERIALS AND METHODS

Experimental diets

A 3 × 3 factorial experiment in a completely randomized design using three replicates was used. Nine experimental diets were formulated to contain with three levels of protein (35, 40 and 45%) and three levels of energy (3.8, 4.0 and 4.2 kcal g^{-1}). The feed formulation and biochemical composition of the experimental diets are summarized in Table 1. The major dietary protein sources used were fish meal, shrimp meal and soybean meal and dietary protein level increased in a proportion to its percentage. Wheat flour was used as carbohydrate source and tuna oil was used as the lipid source. Mineral and vitamin mixes were added to the diets. Wheat gluten was used as binders. The diets were prepared by weighing the dry ingredients and mixing thoroughly in a mixer. The lipid sources were added drop by drop while the mixture was further blended to ensure homogeneity. Approximately 200 ml hot water was then added for each kg of this mixture. The diets were extruded and dried at room temperature for 48 h. Upon feeding, the feeds were made into small pieces (round shape of 1.5 cm diameter) to facilitate sucking by the snails. All experiment diets were then stored in a refrigerator at 4°C until use. All diets were analyzed in duplicate for the proximate compositions according to standard methods (AOAC, 1990).

Pond preparation and culture management

This study was assigned to use the recirculating culture system. A series of rectangular plastic tanks (1.0 × 3.0 × 1.0 m) were used as the rearing ponds and the animals were kept in rearing unit (plastic baskets) of 25.0 × 35.0 × 25.0 cm which contained numerous pores of 1.5 cm^2 (4 holes cm^{-2}) at each side. Bottom of each rearing unit was covered with coarse sand of 2 cm thickness as substratum. Aeration was provided with an air diffuser. This study consisted of 9 feeding treatments, and each treatment included three replicates. Twenty-seven baskets were assigned to the tanks using a completely randomized design. A plastic tank (3.0 × 2.0 × 1.0 m) was used as the biological filter tanks containing bioballs as biofilter. Seawater from the rearing pond was flowed into the biological filter tank and seawater was pumped back into the rearing pond continuously at a constant rate of 200 L/h. Rearing units were scrubbed and new seawater in rearing pond was replaced every 30 days after measurement of length and weight to minimize accumulation of metabolites in the culture system. During the experimental period, water quality was maintained periodically during the feeding trials. Water temperature and salinity were 29.0 - 31.0°C and 29.0 - 30.0 ppt, respectively. Salinity was monitored daily, as necessary, to keep the variation within ±2.0 ppt by addition of fresh water to correct for any increased salinity due to water evaporation. Dissolved oxygen was not less than 5 mg/l, and there were negligible levels of free ammonia and nitrite. Natural light cycle was used in the feeding trials.

Experimental animals and feeding experiments

Juvenile *B. areolata* used in the feeding trials were transported from a commercial private hatchery in Petchaburi, Thailand, transported to the laboratory and kept in three 300 L circular plastic tanks for acclimatization. During the acclimatization period, the snails were fed chopped trash fish mixed with the basal diet. The amount of trash fish was gradually replaced by the diet until the snails can accept diet totally. The acclimatization period lasted over 5 days. At the beginning of the experiment, healthy juveniles were sorted a uniform size to prevent possible growth retardation of small spotted Babylon when cultured with larger ones. Mean initial shell length and whole body weight of juveniles were 1.15 ± 0.01 cm and 0.29 ± 0.01 g (mean ± S.D, n = 30), respectively and did not differ significantly ($p > 0.05$) among treatments (Table 2). Juveniles were distributed randomly into 27 rearing tanks of 25.0 x 35.0 x 25.0 cm (3 tanks /diet) at a density of 50 snails per tank. At the beginning of the feeding trial, juveniles were hand-fed to once daily (10:00 h) to apparent visual satiation with the experimental diets. All groups were fed their respect diets at the same fixed rate of initially 5% of body weight per day. The amount of feed was adjusted daily based on the amount of food consumed by snails within 0.5 h on the previous day to ensure that only a minimal amount of feed left. Apparent satiation was determined from observation of the point at which snails ceased active feeding, moving away from the feeding area and buried under sand substratum. Uneaten food was siphoned out immediately after the snails stopped eating to prevent contamination of water and sand substratum. The amount of feed eaten was recorded daily for calculation of feed conversion ratio. No any chemical and antibiotic agent was used throughout the entire experimental periods. Grading by size was not carried out in any pond throughout the growing - out period. Mortalities were recorded daily. Each feeding trial was terminated at 16-week.

Sample collection and analysis

The wet weight of all snails from each tank was measured individually at the beginning of the experiment and every 2 weeks in feeding trials for growth estimation. Growth performance and feed utilization was calculated as following: specific growth rate (SGR), absolute growth rate (AGR), feed efficiency (FE) and survival. The calculation formulae are as following: percent weight gain (PWG, %) = [(final weight - intial weight) / initial weight] x 100; specific growth rate in weight (SGRW, % day^{-1}) = [(ln final body weight - ln initial body weight) / feeding trial period in day] x 100; feed conversion ratio (FCR) = dry feed fed (g) / wet weight gain (g); and survival (SR, %) = 100 x (final snail number) / (initial snail number) (Tan, Mai and Luifu, 2001; Ye et al., 2006; Liu et al., 2006).

Statistical analysis

The data are presented as mean ± SD of the three replicates. All growth data from each treatment were analyzed by one-way and two-way analysis of variance (ANOVA) to test for the effects of the dietary protein and energy levels. If significant ($p < 0.05$) differences were found in the one-way ANOVA test. A least significant difference test was used to compare means. Treatment effects were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Final weight (FW), weight gain (WG), specific growth rate in weight (SGR), survival rate (SR) and feed conversion ratio (FCR) of juvenile *B. areolata* fed experimental diets (Figure.1) containing various dietary protein (DP) and dietary energy (DE) levels for 16 weeks are presented in Table 2. Two-way ANOVA showed that dietary protein significantly affected FW, WG and SGR ($P < 0.05$) but not for SR, while dietary energy did not affect FW, WG, SGR and SR ($p > 0.05$) but not for FCR. Significant interactions were observed between dietary protein and energy levels ($p < 0.05$) regarding FW, WG, SGR and FCR but not for those of SR (Table 2). After 16 weeks of the feeding trials, the spotted Babylon fed the diet containing 35% DP and 4.0 kcal/g had the highest final weight (2.88 g/snail), which were significantly different from those fed 45% DP diets at all dietary energy levels (2.17 - 2.67 g/snail). Weight gain (WG) of spotted Babylon was significantly influenced by dietary protein levels ($p < 0.05$) but not for dietary energy level ($P > 0.05$) and WG of snails were improved as dietary protein decreased ($p < 0.05$). However, there was no significant difference in WG between those of snails fed the diet containing 35% and 40% dietary protein at the same energy levels ($p > 0.05$). Spotted Babylon fed diet 2 (35% DP and 4.0 kcal g^{-1} DE) had the highest WG (2.58 g / snail). For all dietary protein, PWG of snails increased with increasing dietary energy levels. Spotted Babylon fed diet 2 (35% DP and 4.0 kcal g^{-1} DE) had the highest PWG (874.53%), while those fed diet of 45% DP at all

Table 2. Weight gain, specific growth rate, survival rate, feed efficiency ratio and protein efficiency ratio of juvenile *B. areolata* fed experimental diets containing different dietary protein and energy levels for 16 weeks.

Diet	CP	DE	Final Weight (g)	WG[1](mg/snail)	PWG[2] (%)	SGRW[3](%day^{-1})	SR[4] (%)	FCR[5]
1	35	3.8	2.19 ± 0.11^{a}	1.89 ± 0.11^{a}	651.29 ± 28.91^{a}	1.88 ± 0.09^{a}	98.0 ± 1.2^{a}	4.54 ± 0.23^{a}
2	35	4.0	2.88 ± 0.05^{b}	2.58 ± 0.06^{b}	874.53 ± 10.21^{b}	2.14 ± 0.02b	98.6 ± 0.7^{a}	3.21 ± 0.11^{b}
3	35	4.3	2.58 ± 0.34^{c}	2.29 ± 0.35^{c}	759.54 ± 29.15^{c}	2.05 ± 0.17^{b}	98.0 ± 3.6^{a}	4.10 ± 0.43^{c}
4	40	3.8	2.65 ± 0.23^{d}	2.36 ± 0.24^{d}	787.61 ± 64.87^{d}	2.08 ± 0.11^{b}	97.5 ± 2.9^{a}	3.88 ± 0.29^{d}
5	40	4.0	2.58 ± 0.12^{c}	2.29 ± 0.12^{c}	816.72 ± 73.92^{e}	2.07 ± 0.07^{b}	98.2 ± 0.6^{a}	3.62 ± 0.20^{e}
6	40	4.3	2.67 ± 0.29^{d}	2.38 ± 0.27^{d}	829.30 ± 99.24^{f}	2.09 ± 0.11^{b}	98.3 ± 10.9a	3.68 ± 0.50^{f}
7	45	3.8	2.34 ± 0.07^{e}	2.05 ± 0.08e	696.90 ± 63.72^{g}	1.94 ± 0.07^{ab}	97.7 ± 7.8^{a}	4.44 ± 0.24^{a}
8	45	4.0	2.17 ± 0.04^{a}	1.89 ± 0.04a	656.59 ± 32.21^{a}	1.88 ± 0.05^{a}	97.8 ± 10.4^{a}	4.43 ± 0.14^{a}
9	45	4.3	2.17 ± 0.07^{a}	1.85 ± 0.07^{a}	579.73 ± 34.81^{h}	1.85 ± 0.08^{a}	97.8 ± 7.3^{a}	4.54 ± 0.08^{a}
Two – way ANOVA								
Dietary protein (CP)			0.00	0.000	0.000	0.000	0.442	0.000
Dietary energy (DE)			0.132	0.132	0.037	0.162	0.519	0.001
CP x DE			0.001	0.001	0.003	0.007	0.717	0.003

Values (means ± SD of three replications) in the same column with different superscript are significantly different ($P < 0.05$).
CP: Crude protein (%); DE: Gross energy (kcal g^{-1})
[1] weight gain: (final weight – intial weight)
[2] Percent weight gain: [(final weight – intial weight) / initial weight] x 100
[3] Specific growth rate in weight: 100 (ln final weight – ln initial weight) / days
[4] Survival: 100 (final snail number) / (initial snail number)
[5] Food conversion ratio: Feed consumed / wet weight gain.

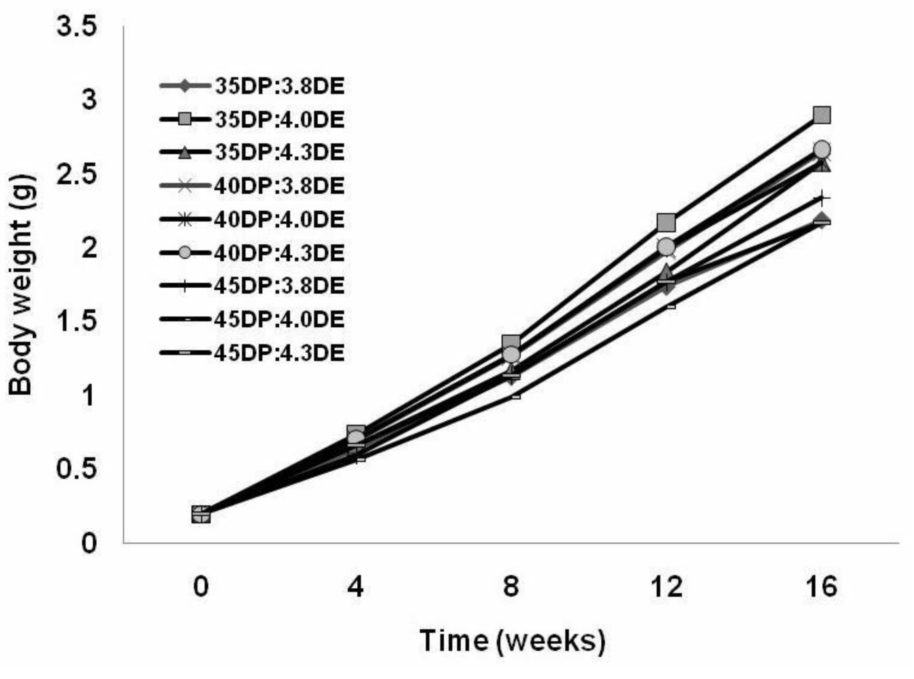

Figure 1. Growth in body weight of juvenile *B. areolata* fed experimental diets containing different dietary protein and energy levels for 16 weeks.

dietary energy levels had the lowest PWG (579.73 - 696.90%), which were significantly different from those fed other DP and DE diets (ranging 651.29 - 829.30%).

Similarly, specific growth rate in weight (SGRW) of spotted Babylon was significantly influenced by dietary protein levels ($p < 0.05$) but not for dietary energy levels

($p > 0.05$) and SGRW of snails was improved as dietary protein decreased ($p < 0.05$). However, there was no significant difference in SGRW between those of snails fed the diet containing 35% and 40% protein at the same energy levels ($p > 0.05$). Spotted Babylon fed diet 2 (35% DP and 4.0 kcal g^{-1} DE) had the highest SGRW (2.14% day^{-1}), which were not significantly different from those fed diet 3, 4, 5 and 6 (ranging 2.05 - 2.09% day^{-1}), but not for those fed diet 1, 7, 8 and 9 (ranging 1.85 - 1.94 % day^{-1}). This study did not agree with the study of Zhou et al. (2007) which mentioned that optimal dietary protein requirement for maximum growth and feed utilization of juvenile *B. areolata* is 45% of dry diet. Maximum weight gain, specific growth rate and soft body to shell ratio occurred at 43% dietary protein level. However, those diets contained higher levels of energy than all diets used in this study, ranging 15.13 to 15.40 MJ kg^{-1}. Furthermore, Lee and Kim (2005) showed that mean weight gain of the snail *Semisulcospira gottschei* was improved with increasing dietary protein level up to 22 and 32% at 3.3 and 3.9 kcal g^{-1} diet, respectively, and mean weight gain of snails fed the 22% protein diet with 3.9 kcal g^{-1} diet was not significantly different from that of snails fed the 32 - 52% protein diets with 3.3 and 3.9 kcal g^{-1} diets. Considering this growth response, optimal dietary protein and energy levels are about 35% and 4.0 kcal/g diet for growth of the spotted Babylon.

Survival rates (SR) of each group was all above 95% and no significant difference among dietary treatments ($P > 0.05$). SR of spotted Babylon was not influenced by dietary protein and dietary energy levels, which agreed with the studies with various fish and shellfish such as snail *Semisulcospira gottschei* (Lee and Kim 2005), abalone *Haliotis asinina* (Teruel and Millamema, 1999), olive flounder *Paralichthys olivaceus* (Kim et al. 2004), cuneate drum *Nibea miichthioides* (Wang et al. 2006), rock lobster *Jasus edwardsii* (Ward et al. 2003), ivory shell *Babylonia areolata* (Zhou et al. 2007), native frog *Rana rugulosa* (Somsueb and Boonyaratpalin, 2001), yellowtail *Seriola dumerili* (Vidal et al., 2008). Survival was generally high ranging 97.8 - 98.6% for all treatments. The high survival of spotted Babylon noted for all treatments may well indicated that there was generally a balance of nutrients in the diets although the lower protein content in the diet may not have been enough to sustain comparable growth of spotted Babylon with those fed the other dietary protein and energy diets.

Feed conversion ratio (FCR) of spotted Babylon was significantly influenced by dietary protein and dietary energy levels ($p < 0.05$). Diets containing 35% dietary protein and 4.0 kcal / g dietary energy appeared to be utilized more efficiently in term of FCR (3.21) than diets containing the other dietary protein and energy levels, ranging 3.62 - 4.54. The FCR (>4.0) shown in Table 2 obtained with spotted Babylon fed the highest protein diet (45%) at all dietary energy, ranging 4.43 - 4.54). However, the FCR obtained with spotted Babylon fed 40% protein diet at all dietary energy ranged 3.62 - 3.88. This result suggested that the amount of calories taken in by the snails somehow affected the amount of food consumed.

The protein and energy requirements of aquatic animals vary with fish and shellfish species size, dietary protein quality, and environmental conditions. The non protein energy levels may also influence the dietary protein requirement of animals. When insufficient non-protein energy is available in feeds, dietary protein is deaminated in the body to supply energy for metabolism rather than being used for tissue growth, and excreted ammonia can reduce water quality. Because fish consume food to satisfy their energy requirement, excess dietary energy may limit intake of essential nutrients like protein and amino acids. Thus, excesses of energy can lead to growth reduction and increase fat deposition in fish (Daniels and Robinson 1986). A good agreement was observed in this study that the snails fed on diets containing higher protein level (45%) and energy levels 94.0 and 4.5 kcal/g diet had slower growth than those fed diets with lower protein of 35% and 4.0 kcal/g diet of energy level. Lee and Kim (2005) also indicated that a diet containing 22% protein and 3.3 kcal/g diet with P/E ratio of 69 mg protein/kcal was recommended for growth of snail (*Semisulcospira gottschei*). In addition, snails fed the 3.9 kcal/g diet showed a tendency toward to higher in 18:1n-9, 18:2n-6, 18:3n-3 and 22:6n-3 and lower in 20:4n- 6 and 22:1n-9 than those of snails fed the 3.3 kcal/g diet energy diets. He also suggested that snail require n-3 unsaturated fatty acids as essential fatty acids in diets for normal growth, and plant oil could be used as an energy source when n-3 highly unsaturated fatty acids requirement is satisfied. Information on essential fatty acids requirements of spotted Babylon is not available. Some studies showed that the proper ratio of n-3 to n-6 levels was critical for the growth or high resistance to disease infection of several fish rather than n-3 level or n-6 level alone (Lee and Kim, 2001). Therefore, more studies considering dietary fatty acid profiles on the performance of spotted Babylon are necessary. Furthermore, if the most effective protein / energy ratio is maintained, then a reduction in the level of dietary protein without a corresponding reduction in growth might be possible. Also, a modification of the proportions of dietary carbohydrate and lipid may contribute to a greater growth response. However, a possible reduction in dietary lipid, as suggested by the efficient use of carbohydrate as an energy source must be approached carefully to ensure that satisfaction of the essential fatty acid requirements is maintained. A reduction in dietary lipid will also contribute to successful manufacture of a diet on a commercial scale.

In conclusion, results of the present study indicate that a diet containing 35% dietary protein and 4.0 kcal g^{-1} diet with P/E ratio of 85.99 mg protein / kcal was recommended for juvenile *B. areolata* growth under our experimental recirculating seawater conditions.

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