ผลของการเสริมสาหร่ายสีน้ำตาล (*Ascophyllum nodosum*) ในอาหาร ต่อการเจริญเติบโตของหอยหวาน (*Babylonia areolata*)

Effect of Brown Seaweed (*Ascophyllum nodosum*) Supplement in Diet on Growth Performance of Spotted Babylon (*Babylonia areolata*)

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บทคัดย่อ

มีการศึกษาวิจัยเกี่ยวกับการใช้ประโยชน์จากสาหร่ายสีน้ำตาล (*Ascophyllum nodosum*) ในการเพาะเลี้ยงสัตว์ ซึ่งมีผลต่อการเจริญเติบโต การเสริมภูมิคุ้มกัน และการต้านโรค ของกุ้ง และปลาหลายชนิด ซึ่งครั้งนี้เป็นครั้งแรกในการศึกษาผลของสาหร่ายต่อการเลี้ยงหอย จากการศึกษาพบว่าการเสริมสาหร่าย *A. nodosum* ลงในอาหารมีผลดีต่อการเจริญเติบโตของหอยหวาน (*Babylonia areolata*) โดยแบ่งหอยหวานเป็นสี่กลุ่ม สามซ้ำ เลี้ยงด้วยอาหารที่ผสมสาหร่ายสีน้ำตาล สี่สูตรคือ ผสมสาหร่ายร้อยละ 0 ซึ่งเป็นชุดควบคุม ร้อยละ 2.5 ร้อยละ 5.0 ร้อยละ 10.0 โดยน้ำหนัก และปลาสด เป็นเวลา 30 วัน พบว่าการเสริมสาหร่ายสีน้ำตาลให้ผลดีกว่าชุดควบคุม ทั้งด้านความยาวเปลือก และน้ำหนักเปียก อย่างมีนัยสำคัญทางสถิติ ($P<0.05$) แต่ให้ผลน้อยกว่าปลาสด การศึกษาครั้งนี้แสดงให้เห็นถึงศักยภาพของการใช้สาหร่ายเคลป์เป็นอาหารเสริม และควรมีการศึกษาในด้านต่าง ๆ อย่างต่อเนื่อง เพื่อประยุกต์ใช้ในการเลี้ยงหอยหวาน

Abstract

Brown seaweed (*Ascophyllum nodosum*) applications on aquaculture have been studied in previous researches those presented effects on growth performance, immuno-stimulating and diseases resistance in many species of shrimp and fish. This research is the pioneer study of brown seaweed application in mollusk species. Brown seaweed (*A. nodosum*) meal supplement in diet presented the good effect on growth performance of spotted babylon (*Babylonia areolata*). The young *B. areolata* were divided into five groups with triplicates. All groups were fed for 30 days with *A. nodosum* meal supplement in artificial diet 0% as control diet, 2.5%, 5.0%, 10.0% (w w^{-1}) and fish meat. The result of *A. nodosum* supplement showed better effect than control diet both on total shell length and wet whole body weight with significant difference ($P<0.05$) but lower than fish meat. This

study presents the potential of *A. nodosum* as diet supplement and should be continuously studied for multi-application of *B. areolata* culture.

1. Introduction

Spotted Babylon (*Babylonia areolata*) is the new economical marine snail of Thailand and exportation. At the present, *B. areolata* quantity is rapidly reducing to the critical level and its size is smaller than the past while the marketable requirement is rapidly increasing. So, the research of *B. areolata* cultural techniques is importance for the production increment and stock enhancement.

Brown seaweed such contains a range of different polysaccharides including alginate, larminarans and fucoidan those are currently subjects of much research interest to characterize their structures and understand their biological activities and application potential in many fields. Particularly interesting is the unique presence of fucoidan, a family of polysaccharides that have immunological stimulating properties in animals and shows much promise in diseases control and other feed applications. The inclusion rate of *A. nodosum* meal in shrimp feed should be around 3.5 to 4%. The average level of fucoidan in *A. nodosum* is around 5% on dry weight basis (Takahashi *et. al*., 1998). This research is the basic knowledge of *A. nodosum* application on growth performance in *B. areolata*.

2. Materials and Methods

2.1 Dietary Preparation

The experimental diets ingredient is shown in Table 1 (modified from Chungthanawong, 2004). The ingredient was mixed by blender and dried at 60°C for 12 h. The three levels of *A. nodosum* meal (0%, 2.5%, 5.0% and 10.0%) were added in basal diet. All experimental diets were stored at 4°C until future use. This dietary formula is primarily done under laboratory conditions. The composition of experimental diets may not show high efficiency on growth of *B. areolata*. The growth performance efficiency comparison between artificial diets and fish was studied. The fresh fish meat, short body mackerel (*Rastrelliger neglectus*); as common feed of *B. areolata* culture was used in this study.

The proximate analysis of all experimental diets was determined following methods of Association of Official Analytical Chemists (AOAC, 1996). The stability of all dietary formulas and fish meat was also tested. The pieces of each diet were soaked in water until those completely decayed that duration was estimated to the stability period of the diets.

Table 1 Ingredient of artificial diets for the experiment

Ingredients	% (w w^{-1})				Fish Meat
	Basal Diet	Formula 1	Formula 2	Formula 3	
R. neglectus	-	-	-	-	100%
Fish Meal	40	40	40	40	-
Soybean Meal	5	5	5	5	-
Wheat Flour	25	22.5	20	15	-
Shrimp Head Meal	5	5	5	5	-
Wheat Gluten	16	16	16	16	-
Fish Oil	5	5	5	5	-
Vitamin Mixture	2	2	2	2	-
Mineral Mixture	2	2	2	2	-
A. nodosum Meal	0	2.5	5	10	-

2.2 Experimental *B. areolata*

The juvenile *B. areolata* was obtained from The Research and Technology Transfer Unit of the Spotted Babylon (*Babylonis areolata*) Station, Aquatic Resources Research Institute, Chulalongkorn University, Pechaburi Province. The average range of the total shell length and wet body weight of *B. areolata* is between 0.8 to 1.3 cm and 1.10 to 1.20 g, respectively. The *B. areolata* was fed with *R. neglectus*, acclimatized to ambient laboratory culture condition. *B. areolata* was fed once daily and uneaten food was removed after the feeding. When the experiment was started, the healthy *B. areolata* were selected from the acclimatized stock and transferred to the experimental tanks. The initial average of total shell length and wet body weight were recorded.

2.3 Experimental Cultural Unit and Facilities

The high concentrate sea water of the salt farm was used as rearing water in this experimental cultural unit. The salt water was diluted to 30‰ and stocked for three days before use. The rearing water qualities were monitored along this experimental period and removed about 60% daily. Clearly circular plastic tanks (20 cm Ø × 30 cm H) were used as rearing tanks. Sandy material was not filled into the tank. The air supply was set and opened in every cultural tank.

2.4 Growth Performance Experiment of *B. areolata*

150 healthy *B. areolata* were divided into five groups with triplicates at 10 *B. areolata* per tank. Each group was fed on 30 days with *A. nodosum* meal supplement in diet 0%, 2.5%, 5.0% and 10.0% (w w^{-1}) and *R. neglectus*. They were fed once daily, uneaten food and fecal matter were removed by siphon after feeding and the rearing water was removed about 60% daily. The growth parameters were calculated as follows (Zhou *et al*., 2007, Brett, 1979 and Sang-ngam, 1998):

$$\text{Specific growth rate, SGR} = \frac{(\ln \text{final size} - \ln \text{initial size}) \times 100}{\text{number of feeding day}} \quad \text{.....(1)}$$

$$\text{Feed conversion ratio, FCR} = \frac{\text{weight of feed}}{(\text{final weght} - \text{initial weght})} \quad \text{.....(2)}$$

$$\text{Percent Weight gain, WG} = \frac{\text{final weight} \times 100}{\text{initial weight}} \quad \text{.....(3)}$$

$$\text{Percent Shell length increase, SLI} = \frac{(\text{final shell length} - \text{initial shell lelngth}) \times 100}{\text{initial shell elngth}} \quad \text{.....(4)}$$

2.5 Water Qualities Determination

The major qualities of the rearing water were measured every day along the experimental period. Salinity was measured by Hand-Held Refractometer (Atago®), temperature was measured by handle thermometer, pH was measured by indicator paper, dissolve oxygen (DO), total ammonia and total alkalinity were measured by test kits (AQUA–VBC®).

2.6 Statistical Analyses

All of the experiments in this study were set on completely randomized design (CRD). All parameters were statistical evaluated using analysis of variance at level of significance 0.05, homogeneity of variance and multiple comparisons by Duncan's New Multiple Range Tests at confidence interval 95% (Sang-ngam, 1998 and Sokal and Rohlf, 1981).

3. Results

3.1 Experimental Diets

The result of proximate analysis of experimental diets is shown in Table 2. In four artificial diets, the basal diet is the highest average of protein percentage and significant difference (P>0.05). The average of lipid percentage is no significant difference (P>0.05). The *A. nodosum*

10% formula is the highest average ash, moisture and fiber percentage. The stability of all experimental diets is approximately 2.0 h. The fish meat showed different results.

Table 2 Dietary proximate analysis of the experiment

Diet Formulas	Contents					Stability
	Protein (%)	Lipid (%)	Ash (%)	Moisture (%)	Fiber (%)	(h)
Basal Diet	44.30 ± 0.03[a]	10.48 ± 0.17[b]	12.84 ± 0.14[d]	7.52 ± 0.01[a]	2.62 ± 0.02[c]	2
A. nodosum 2.5%	43.92 ± 0.07[ab]	10.48 ± 0.09[b]	13.60 ± 0.17[c]	7.28 ± 0.06[a]	2.73 ± 0.13[bc]	2
A. nodosum 5.0%	43.78 ± 0.01[ab]	10.34 ± 0.11[b]	15.37 ± 0.05[b]	7.23 ± 0.05[a]	2.84 ± 0.06[b]	2
A. nodosum 10.0%	43.56 ± 0.03[b]	10.31 ± 0.04[b]	16.21 ± 0.13[a]	7.18 ± 0.02[a]	3.11 ± 0.12[a]	2
Fish Meat	19.07 ± 0.53[c]	12.63 ± 0.57[a]	1.39 ± 0.13[e]	67.04 ±1.64[b]	>0.01 ± 0.00[d]	>2

Note: Mean ± SD (n=3) and the same superscript indicates non significant difference (P>0.05)

3.2 Efficiency of *A. nodosum* Meal Supplement in Diet on the Growth of *B. areolata*

The results of *A. nodosum* meal supplement in diet on the growth of *B. areolata* are shown in Tables 3 and 4. The means of total shell length and wet body weight of *B. areolata* are significant difference (P<0.05). Fish meat showed the highest average of total length, wet body weight, FCR, SLI, SGR and WG. *A. nodosum* meal supplement 2.5% in diet exhibited high of all parameters than other formulas. Survival rate of *B. areolata* was 100% in all dietary treatments which not showed significant effect on survival rate.

3.3 Water Qualities

The major qualities of the rearing water were determined everyday. The range of temperature was 27 to 28°C, salinity was 27 to 30‰, pH was 8.0 to 8.5, dissolved oxygen (DO) is 6 to 8 mg L^{-1}, total ammonia was 0.00 to 0.2 and alkalinity was 150 to157 mg L^{-1}

Table 3 Growth on total shell length and wet body weight of *B. areolata* with different diets

Diet Formulas	Total Length (mm)		Wet Weight (g)		Survival
	Initial	Final	Initial	Final	Rate (%)
A. nodosum 0%	18.10 ± 0.24	21.11 ± 0.34[c]	1.18 ± 0.13	2.17 ± 0.03[b]	100
A. nodosum 2.5%	18.10 ± 0.24	21.23 ± 0.20[b]	1.18 ± 0.13	2.17 ± 0.02[b]	100
A. nodosum 5.0%	18.10 ± 0.24	21.17 ± 0.20[bc]	1.18 ± 0.13	2.14 ± 0.02[c]	100
A. nodosum 10.0%	18.10 ± 0.24	21.11 ± 0.16[c]	1.18 ± 0.13	2.10 ± 0.06[d]	100
Fish Meat	18.10 ± 0.24	22.61 ± 0.14[a]	1.18 ± 0.13	2.98 ± 0.12[a]	100

Table 4 Growth indexes of *B. areolata* with different diets

Formulas	SGR (%/day)		FCR	SLI (%)	WG (%)
	Total Length	Wet Weight			
A. nodosum 0%	0.51 ± 0.05[bc]	2.03 ± 0.05[b]	2.03 ± 0.06[e]	16.63 ± 1.89[c]	184.02 ± 2.71[b]
A. nodosum 2.5%	0.53 ± 0.03[b]	2.03 ± 0.03[b]	2.06 ± 0.04[d]	17.31 ± 1.01[bc]	183.67 ± 1.59[b]
A. nodosum 5.0%	0.52 ± 0.03[bc]	1.99± 0.03[c]	2.09 ± 0.04[c]	16.96 ± 1.01[c]	181.50 ± 1.45[c]
A. nodosum 10.0%	0.51 ± 0.02[c]	1.92 ± 0.02[d]	2.18 ± 0.03[b]	16.59 ± 0.89[c]	178.14 ± 1.16[d]
Fish Meat	0.74 ± 0.02[a]	3.10 ± 0.01[a]	3.86 ± 0.03[a]	24.90 ± 0.77[a]	252.91 ± 1.04[a]

Note: Mean ± SD (n=30, triplicate) and the same superscript indicates non significant difference (P>0.05). Initial weight was about 1.18 ± 0.13 g and initial shell length was 18.10 ± 0.24 cm. SGR: specific growth rate, FCR: feed conversion ratio, SLI: shell length increase, WG: weight gain

4. Discussion

4.1 Experimental Artificial Diets

The proximate analysis of all experimental artificial dietary formulas showed high level of protein content. The high protein formula showed good effect on survival and growth of healthy *B. areolata* than low protein formula (Sang-ngam, 1997). The high *A. nodosum* level formula showed high ash and fiber content that may be affected on *A. nodosum* content (Chungthanawong, 2004). The stability of the experimental diets for 2 h is long enough for babylon feeding.

4.2 Efficiency of *A. nodosum* Meal Supplement in Diet on the Growth of *B. areolata*

The effect of seaweed on growth performance has been studied in pig (Turner *et al*., 2002), calve (Evans *et al*., 2002), fish (Valente *et al*., 2006 and Davies *et al*., 1997) and shrimp (Chungthanawong, 2004), but no on mollusk species. In this study, *A. nodosum* supplement showed the effect on growth of *B. areolata*. In terms of total shell length, the *B. areolata* fed with *A. nodosum* 2.5% and 5.0% dietary formulas were significantly longer than those from control and 10.0% formulas. In terms of wet weight, *B. areolata* which were fed with *A. nodosum* 0% and 2.5% dietary formulas were significantly heavier than those from 5.0% and 10.0% formulas. However, *A. nodosum* meal supplement 0% and 2.5% were no significant difference on total shell length and wet body weight. In particular, there was no importance for *A. nodosum* meal supplement in diet because *A. nodosum* exhibited unclear effect on growth performance of *B. areolata.*

These results showed that the growth gain of juvenile *B. areolata* increased with high protein of artificial diets. These results are similar to those in other mollusk species (Uki *et al*., 1986, Mai *et al*., 1995). On the basis of this study, 45% protein in artificial diet may be recommended for growth of *B. areolata*. The optimal protein level for abalone was between 20% and 35% (Uki and Watanabe 1992). These differences might be that *B. areolata* was a carnivorous or scavenger mollusk whereas abalone was omnivorous or herbivorous.

The *B. areolata* fed with fish meat showed the highest growth than other formulas since fish meat was an appropriately feed. The artificial diet is important for intensive aquaculture in long term due to the production of fish being not stable on market supply and can be designed to many requirements. Thus the research on artificial diet of *B. areolata* should be continuously developed.

4.3 Water Qualities

All water parameters along the experimental period were rather stable because the water was exchanged 60% daily after feeding. The qualities of water were in the standard range for aquaculture and the culture density was suitable for the growth of juvenile *B. areolata* (Liu and Xiao, 1998 and Chaitanawisuti and Kritsanapuntu 2002).

5. Conclusion and Recommendation

The *B. areolata* feeding high level of *A. nodosum* supplement in artificial diet exhibited bad significant effect on growth of *B. areolata* than those feed low level or none of *A. nodosum* supplement. *A. nodosum* may not be an effective diet material on growth performance but may be an effective in other properties of *B. areolata*. This research is the pioneer study on the potential utilization of *A. nodosum* application in *B. areolata* culture. The related fields of this study should be continued studying such as chemistry, biochemistry and bioactivity of *A. nodosum*.

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