

# Effects of Dietary Partial Replacement of Tuna Oil by Corn Oil in Formulated Diets for Growth Performance and Proximate Composition of Juvenile Spotted Babylon *Babylonia areolata* under Hatchery Conditions

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## Abstract

A 120-day feeding experiment was conducted to determine the effects of dietary partial replacement of tuna oil by corn oil at different ratios in formulated diets, by which growth performance and proximate composition of juvenile spotted babilon *Babylonia areolata* was analyzed. Four isonitrogenous and isolipidic diets were formulated with 10% lipid derived from the following lipid sources: diet A (100% tuna oil without corn oil (control), diet B: 50% tuna oil and 20% corn oil, diet C: 30% tuna oil and 40% corn oil and diet D: 10% tuna oil and 60% corn oil. This study showed that all formulated diets were well accepted by the snails throughout the culture period. There were no significant differences in body weight gain, shell length increment and growth rate among the feeding experiments. No significant differences were observed in feed conversion ratio among the dietary treatments ranging from 4.35 to 4.77. Survival was high ranging from 98.00% to 100% and no significant differences in survival were observed. At the end of the experiment, crude protein and fat contents of whole body weight of snails were not statistically different among the feeding experiments. Crude protein and fat of snails fed four experimental diets ranged from 61.38 to 61.48% and 5.36% to 5.39%, respectively. This study indicated partial replacement of tuna oil by corn oil in formulated diets have no effects on growth performance but fat content of the whole body reduced to half than those contained in formulated diets.

**Keywords:** *B. areolata*; Formulated diet; Fish oil; Corn oil; Growth; Survival; Food efficiency

## Introduction

Traditional culture of spotted *Babylon Babylonia areolata* in Thailand mainly depended on chopped trash fish as the main feed source for grow out, which is expensive, has limited supply, uncertain nutritional quality, difficult to store, deteriorates water quality and may result in the spread of disease [1]. The establishment of a successful aquaculture industry based on this species requires optimization of their diet for fast growth, high survival, good food efficiency, and low cost, whilst providing better biochemical composition in the flesh. The development of a practical diet for improving the growth performance of this species is seen as one of the key steps for the successful establishment of viable spotted Babylon aquaculture venture in Thailand. Then, it is important to develop a cost-effective and nutritionally balanced feed formulation for this species in the future [2]. Dietary lipid plays a major role in providing a source of concentrated energy for growth and as carriers for fat soluble vitamins. Fish oil contained high quantities of n-3 HUFA and other essential fatty acids necessary for marine fish and shellfish. They serve as a functional element maintaining metabolism and contain attractants that enhance diet palatability [3]. The demand of fish oils in aquafeeds has dramatically increased and has placed unsustainable pressure on this finite resource. In the future, global fish oils production may not be enough to supply the increasing demand of animal feed. Therefore replacement of fish oil with vegetable oils appears to be a viable option given their availability, low cost and absence of diotoxins and pollutants. Thus, the partial replacement of fish oils with vegetable oils in formulated feeds has gained increasing interest from aquaculturists [3-6]. Vegetable oils constitute promising candidates for fish oil replacement, having steadily increasing production, with high availability and better economic value. Some vegetable oils such as soybean oil and rapeseed oil are considered as possible alternative lipid source for freshwater and marine fish [7]. A key requirement

for the replacement of fish oil in aquafeeds is to supply equivalent energy with balanced essential fatty acids. This is necessary in order to sustain high growth, survival, feed conversion efficiency, immune competence, disease resistance, and flesh quality. Hence, this study was designed to determine the effects of partial replacement of tuna oil by corn oil in formulated diets for the growth performance and proximate composition of juvenile spotted Babylon *Babylonia areolata*.

## Materials and Methods

Four isonitrogenous, isoenergetic and isolipidic experimental diets were formulated with 10.3% lipid derived from two lipid sources of tuna oil (TO) and corn oil (CO) as following: diet A (100% TO), diet B (71.5% TO+28.5% CO), diet C (43% TO+57.0% CO) and diet D (14.5% TO+85.5% CO). The ingredients used and proximate composition values of the diets are presented in Table 1. Fish meal served as the main protein source, while tuna oil and corn oil were used as the lipid source (Table 1). The dry ingredients were weighted individually and then thoroughly mixed using an electric grinder. Vitamin and mineral premixes were mixed separately with cellulose prior to mixing with other ingredients. Oils were added to the dry ingredients and mixed

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again. Water was added and the moist mixture was hand-kneaded until it formed stiff dough. It was pelleted using a hand mincer fitted with a 3 mm die, stored in high-gauge plastic bags and preserved in a refrigerator. Proximate analysis of feed was carried out following standard methods [8].

Juvenile *B. areolata* with average initial body weight and shell length of  $0.04 \pm 0.01$  g and  $0.5 \pm 0.01$  cm used in the feeding experiments were purchased from a commercial 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. The acclimatization period lasted over 10 days. At the beginning of the experiment, healthy juveniles were sorted to a uniform size to prevent possible growth retardation of small spotted Babylon when cultured with larger ones. The animals were weighed to the nearest 0.01 g using an electronic balance. Homogenous groups of 1,620 juveniles were distributed randomly into eight rectangular rearing tanks of 55.0×30.0×30.0 cm (3 tanks/diet) at a density of 300 snails per m<sup>2</sup> supplied (50 snails/tank) with flow-through ambient seawater system with a constant flow rate of 150 l per hour. The bottom of the rearing tank was covered with coarse sand as substratum at 1.0 cm thickness. Water depth in rearing tank was 20 cm. The tanks were provided with a supplemental aerator stone and water was aerated daily for 20 h. Light was maintained on a 12:12 h light cycle. Water temperature was maintained at  $29 \pm 2^\circ\text{C}$  by controlling the ambient temperature and salinity held at 30 ppt through the addition of well water. Water quality parameters were as follows: dissolved oxygen >6.50 mg/l, total ammonia nitrogen <0.30 mg/l, nitrite <0.45 mg/l and pH  $\geq 7.8$ . At the beginning of the feeding experiments, juveniles were hand-fed to apparent visual satiation at once daily (10:00 h) with one of the experimental diets. The amount of feed was adjusted daily based on the amount of food consumed by snails within 0.5 h the previous day to ensure that only a minimal amount of feed was left. Satiation was determined from observation of the point at which snails ceased active feeding, moved away from the feeding area and buried under sand substratum. Uneaten food was siphoned out daily after the snails stopped eating to prevent water degradation. The aquaria were cleaned weekly by siphoning 3/4<sup>th</sup> of the water to remove excess feed and faecal matter and by scrubbing the sides of the aquarium. No chemical or antibiotic agent was used throughout the entire experimental period. Grading by size was not carried out in any pond throughout the growing - out period.

Snails were individually weighed every 30 day throughout the experimental period which lasted for 90 days from March to June, 2011. For sampling and data collection, fifty snails were randomly sampled for initial weight and shell length measurement. Weight and shell length measurement was repeated on day 30, 60, 90 and 120 in order to determine growth rate of the snails. At the end of the on-growing period, 12 snails/dietary treatments were sampled for morphometric measurements (total body weight and total shell length) individually. Total body weight and total length were measured. Mortality was determined by counting the number of dead snails and expressing the results as a percentage of the number of snails at the start of the experiment. The following indices of growth were calculated at the end of the experiment: Length increment (LI)=final shell length (cm)-initial shell length (cm); Weight gain (WG)=final body weight (g)-initial body weight (g); Absolute growth rate in weight=(final body weight (g)-initial body weight (g))/T; Absolute growth rate in length=(final shell length (cm)-initial shell length (cm))/T; where T=120 days of culture; Feed conversion ratio (FCR)=feed intake (g)/weight gain (g) and Survival rate (SR)= $100 \times (\text{final snail number}) / (\text{initial snail number})$ .

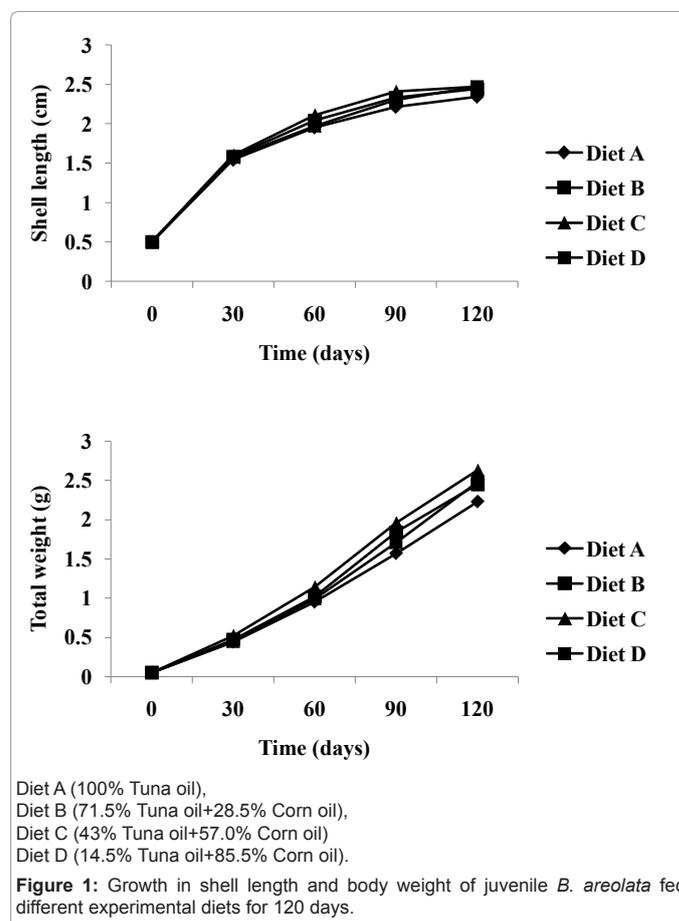
The whole body tissue samples from each dietary treatment were analyzed for proximate composition (crude protein, crude fat, ash and carbohydrate). The tissue samples were minced and a portion removed for moisture and ash content. Moisture was determined by drying pre weighed samples in porcelain cups at 104°C for 24 h, and then ash was determined by incinerating the dried samples at 500°C for 12 h. The rest of the whole tissue was lyophilized and used for crude protein and fat analyses. The protein content was determined by the Kjeldahl method and the fat by Soxhlet extraction with petroleum ether according to the standard methods of AOAC [8]. All analyses were performed in triplicate.

Statistically analyzed data were performed by analysis of variance (ANOVA) using SPSS program, version 11.5. The effect of different lipid sources on growth rate, survival and feed conversion ratio data were subjected to a one-way ANOVA. Duncan's multiple range test was used to compare differences between treatment means when significant F values were observed at P<0.05 level.

## Results

### Growth performance

Growth in body weight and shell length of juvenile *B. areolata* fed on four experimental diets for 90 days was showed in Figure 1. This study showed that all formulated diets were well accepted by the snails throughout the culture period. At the end of the growing-out period, there was no significant differences (P>0.05) in body weight gain, shell length increment and growth rate among the feeding experiments.



Ingredients (g/kg diet)	Diet no. (% tuna oil/% corn oil)			
	Diet A (100/0)	Diet B (71.5/28.5)	Diet C (43.0/57.0)	Diet D (14.5/85.5)
Fish meal	410.0	410.0	410.0	410.0
Soy bean meal	190.0	190.0	190.0	190.0
Shrimp meal	30.0	30.0	30.0	30.0
Wheat	170.0	170.0	170.0	170.0
Wheat gluten	70.0	70.0	70.0	70.0
Vitamin mix <sup>a</sup>	20.0	20.0	20.0	20.0
Mineral mix <sup>b</sup>	20.0	20.0	20.0	20.0
Cellulose	20.0	20.0	20.0	20.0
Tuna oil	70.0	50.0	30.0	10.0
Corn oil	0	20.0	40.0	60.0
Proximate composition (%)				
Crude protein	39.98 ± 0.20	40.04 ± 0.07	40.01 ± 0.04	40.08 ± 0.05
Crude lipid	10.42 ± 0.05	10.38 ± 0.05	10.39 ± 0.04	10.36 ± 0.02
Fiber	2.19 ± 0.06	2.21 ± 0.03	2.21 ± 0.03	2.2 ± 0.03
Water content	13.20 ± 0.04	13.22 ± 0.04	13.24 ± 0.05	13.29 ± 0.02
Ash	14.29 ± 0.07	14.27 ± 0.04	14.29 ± 0.03	14.29 ± 0.05

<sup>a</sup>Vitamin premix (mg kg<sup>-1</sup> or IU): vitamin A, 10000000 IU; vitamin D3, 1000000 IU; vitamin E, 10000 mg kg<sup>-1</sup>; vitamin K3, 1000 mg kg<sup>-1</sup>; vitamin B1, 500 mg kg<sup>-1</sup>; vitamin B2, 5000 mg kg<sup>-1</sup>; vitamin B6, 1500 mg kg<sup>-1</sup>; vitamin C, 10000 mg kg<sup>-1</sup>; folate, 1000 mg kg<sup>-1</sup>; dealthionine, 16038 mg kg<sup>-1</sup>

<sup>b</sup>Mineral premix (mg kg<sup>-1</sup>): Ca, 147 g kg<sup>-1</sup>; P, 147 g kg<sup>-1</sup>; Fe, 2010 mg kg<sup>-1</sup>; Cu, 3621 mg kg<sup>-1</sup>; Zn, 6424 mg kg<sup>-1</sup>; Mn, 10062 mg kg<sup>-1</sup>; Co, 105 mg kg<sup>-1</sup>; I, 1000 mg kg<sup>-1</sup>; Se, 60 mg kg<sup>-1</sup>

**Table 1:** Ingredients and proximate composition of the experimental diet.

Parameters	Diet no. (% tuna oil/% corn oil)			
	Diet A (100/0)	Diet B (71.5/28.5)	Diet C (43.0/57.0)	Diet D (14.5/85.5)
Initial shell length (cm)	0.5 ± 0.01	0.5 ± 0.01	0.5 ± 0.01	0.5 ± 0.01
Initial body weight (g)	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01
Final shell length (cm)	2.34 ± 0.14	2.44 ± 0.02	2.47 ± 0.08	2.38 ± 0.14
Final body weight (g)	2.24 ± 0.03	2.44 ± 0.18	2.63 ± 0.35	2.47 ± 0.46
Shell length increments (cm)	1.84 ± 0.14	1.94 ± 0.02	1.97 ± 0.08	1.88 ± 0.14
Body weight gains (g)	2.24 ± 0.03	2.47 ± 0.22	2.87 ± 0.21	2.47 ± 0.47
Absolute growth rate (cm mo <sup>-1</sup> )	0.46 ± 0.03	0.49 ± 0.05	0.49 ± 0.02	0.47 ± 0.04
Absolute growth rate (g mo <sup>-1</sup> )	0.56 ± 0.05	0.62 ± 0.06	0.72 ± 0.05	0.62 ± 0.12
Feed conversion ratio	4.65 ± 0.21	4.64 ± 0.19	4.77 ± 0.20	4.35 ± 0.17
Final survival rate (%)	98.00	100	98.00	98.00

Diet A (100% Tuna oil),  
Diet B (71.5% Tuna oil+28.5% Corn oil),  
Diet C (43% Tuna oil+57.0% Corn oil)  
Diet D (14.5% Tuna oil+85.5% Corn oil).

**Table 2:** Growth parameters (mean ± SE) of juvenile *B. areolata* fed different experimental diets for 90 days.

Body weight gains were 3.28, 3.36, 3.41 and 3.37 g/snail for snails fed diet 1, 2, 3 and 4, respectively, and those of shell length increments were 1.84, 1.93, 1.98 and 1.87 cm/snail, respectively. The absolute growth rate in shell length were 0.46, 0.49, 0.49 and 0.47 cm/month for snails fed diet 1, 2, 3 and 4, respectively, and those of body weight gains were 0.56, 0.62, 0.72 and 0.62 g/month, respectively (Table 1). No significant differences (P>0.05) were observed in feed conversion ratio among the dietary treatments ranging from 4.35 to 4.77 (Table 2). Survival was high ranging from 98.00% to 100% and no significant differences in survival were observed (P>0.05). The shell length and body weight relationship of juvenile *B. areolata* fed on four experimental diets for 120 days was showed in Table 3.

## Proximate composition

At the end of the experiment, crude protein and fat contents of whole body weight of snails were not statistically different (P>0.05) among the feeding experiments. Crude protein and fat contents of four experimental diets were not statistically different (P>0.05) ranging from 39.98% to 40.08% and 10.36% to 10.42%, respectively (Table 1). However, crude protein and fat contents of whole body weight for snails fed four experimental diets were not statistically different (P>0.05) (Table 4). Crude fat content of whole body is a half less than those contained in formulated diets. Crude protein and fat of snails fed four experimental diets ranged from 61.38 to 61.48% and 5.36% to 5.39%, respectively.

## Discussion

The present study demonstrates that all formulated diets were well accepted by the snails throughout the culture period. There was no significant differences in body weight gain, shell length increment and growth rate among the feeding experiments. No significant differences were observed in feed conversion ratio among the dietary treatments ranging from 4.35 to 4.77. Survival was high ranging from 98.00% to 100% and no significant differences in survival were observed. At the end of the experiment, crude protein and fat contents of whole body weight of snails were not statistically different among the feeding experiments. Crude protein and fat of snails fed four experimental diets ranged from 61.38 to 61.48% and 5.36% to 5.39%, respectively. This study indicated partial replacement of tuna oil by corn oil in formulated diets did not effect on growth performance. This study was agreed with various investigations in marine finfish and shellfish [3,6,7]. Hu et al. [6] reported that there were no significant differences in body weight gain among juvenile *Litopenaeus vannamei* fed practical diets containing different ratios of menhaden oil, soybean oil, and soybean lecithin. Regost et al. [4] reported that replacement of fish oil by vegetable oils (soybean oil and linseed oil) is possible with negligible impact on growth performance of juvenile turbot *Psetta maxima* and dietary lipids are an effect vector to influence the nutritional quality of fish product. Fountoulaki et al. [7] reported that growth and feed utilization in gilthead sea bream *Sparus aurata* are not affected by fish oil substitution with soybean and rapeseed oil, contrary to palm oil

Test diets	Equations	R <sup>2</sup>
Diet A	W=-0.8530 L <sup>1.1201</sup>	0.6550
Diet B	W=-4.0227 L <sup>2.6575</sup>	0.7099
Diet C	W=-4.2884 L <sup>2.7964</sup>	0.7942
Diet D	W=-3.1713 L <sup>2.3802</sup>	0.7179

Diet A (100% Tuna oil),  
Diet B (71.5% Tuna oil+28.5% Corn oil),  
Diet C (43% Tuna oil+57.0% Corn oil)  
Diet D (14.5% Tuna oil+85.5% Corn oil).

**Table 3:** Length and weight relationship of juvenile *B. areolata* fed different experimental diets for 90 days.

Parameters	Diet no. (% tuna oil/% corn oil)			
	Diet A (100/0)	Diet B (71.5/28.5)	Diet C (43.0/57.0)	Diet D (14.5/85.5)
Crude protein (%)	61.38 ± 0.02	61.45 ± 0.01	61.48 ± 0.01	61.39 ± 0.01
Crude lipid (%)	5.36 ± 0.03	5.39 ± 0.01	5.36 ± 0.04	5.37 ± 0.02

Diet A (100% Tuna oil),  
Diet B (71.5% Tuna oil+28.5% Corn oil),  
Diet C (43% Tuna oil+57.0% Corn oil)  
Diet D (14.5% Tuna oil+85.5% Corn oil).

**Table 4:** Nutritional values of whole body tissues of juvenile *B. areolata* fed different experimental diets for 90 days (mean ± SE).

inclusion. Richard et al. [3] concluded that replacing 60% of fish oil by the two mixture of vegetable oils (linseed oil and palm oil) in the feeds of European seabass fingerlings *Dicentrarchus labrax* until they reached the size of 160 g has no marked effect on growth performance, lipogenesis and tissue lipid uptake but has a hypocholesterolemic effect. Peng et al. [5] indicated that complete substitution of fish oil with soybean oil reduced growth efficiency for black seabream *Acanthopagrus schlegelii*. Thus, 60-80% replacement of fish oil by soybean oil is recommended in diet formulation for black seabream. Piedecausa et al. [9] showed that the replacement of fish oil with soybean or linseed oil in sharpnose seabream (*Diplodus puntazzo*) does not affect growth or feed utilization after three months of feeding. Vegetable oils also increased the muscle content of linoleic and linolenic acids. In addition, in terms of economic performance, the soybean oil diet was the least expensive diet, and had the best economic conversion ratio. In conclusion, this study shows that it is possible to substitute almost 50% of fish oil by corn oil in diets for in juvenile *B. areolata*. Fish oil can be replaced by corn oil in juvenile *B. areolata* for a period of 120 days without negatively affecting growth performance and feed utilization. However, it would be interesting to analyze the effects of incorporating this oil in diets for longer periods of time. In addition, more investigation should be continued to reply the question that can vegetable oil replacement at different ratios modify the snail muscle fatty acid profile, reducing/ increasing the levels of EPA, DHA, ARA.

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