Aquaculture
Research & Development

Kritsanapuntu and Chaitanawisuti, J Aquac Res Development 2015, 6:4
http://dx.doi.org/10.4172/2155-9546.1000324

Research Article Open Access

Replacement of Fishmeal by Poultry By-Product Meal in Formulated Diets for Growing Hatchery – Reared Juvenile Spotted Babylon (*Babylonia areolata*)

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Abstract

A feeding trial was conducted to evaluate the effects of five levels of partial to total replacement of fishmeal by poultry by–product meal on growth performance and body composition of hatchery–reared juvenile spotted babylon (*Babylonia areolata*) under aflow-through culture system over 150 days. Five experimental diets were formulated to contain 0%, 25% 50%, 75% and 100% of gradient poultry by-product meal (diet PBM0, PBM25, PBM50, PBM75 and PBM100, respectively). Significant differences ($P<0.05$) in weight gain, specific growth rate, total feed intake, feed conversion ratio, and protein efficiency ratio were found among the feeding treatments, except for final survival rate. Results showed that snails fed diets of PBM25, PBM50, and PBM75 displayed better specific growth rates ranging from 2.19-2.21% day^{-1} and did not differ significantly ($P>0.05$) while snails fed diets of PBM0 and PBM100 showed poorer specific growth rates of 2.03-2.12% day^{-1}, respectively. Final survival rates of the snails ranged from 92.73% -93.94% and did not differ significantly (P>0.05) between feeding treatments. Significant differences ($P<0.05$) were detected in proximate composition (protein, ash, fat, moisture, and carbohydrate, cholesterol content, amino acid composition and fatty acid composition of the whole flesh of experimental snails among all feeding treatment groups. Snails fed diets of PBM-50 resulted in the highest protein and fat contents compared with snails fed the PBM0, PBM25, PBM75 and PBM100 replacement diets. Cholesterol was significantly lower ($P<0.05$) in snails fed diets of PBM75 and PBM100 than in snails fed diets PBM0, PBM25, and PBM50. The whole body composition of snails fed diet of PBM75 was significantly higher ($P<0.05$) in total non-essential amino acids and total essential amino acids than those of snails fed PBM0, PBM25, PBM100, and PBM75. The whole body composition of snails fed PMB-50 was significantly higher ($P<0.05$) regarding EPA, DHA, ARA, n-6 PUFA, and n-3 PUFA contents than those of snails fed PBM0, PBM25, PBM100, and PBM75. The results of this study indicated that poultry by-product meal can replace fishmeal protein by 50-75% with no negative effects in snail growth performance. Moreover, the inclusion of up to 75% poultry by-product meal in the diet improved feed efficiency and body composition.

Keywords: *Babylonia areolata;* Fishmeal; Poultry by-product meal; Growth; Body composition

Introduction

Spotted babylon snails, *Babylonia areolata*, are generally carnivorous feeding mostly on the fresh meat of trash fish. However, feeding fish meat to the snails entails problems with the variability in nutritive content and supply, resulting in a slow and heterogeneous growth rate. Due to problems associated with the use of trash fish as feed for spotted babylon snails, intensive spotted babylon culture is becoming increasingly reliant on formulated practical diets [1]. The use of prepared feeds can be very practical since formulation can be manipulated to obtain an optimum nutritional value. Furthermore, 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 snail production in Thailand [2]. Fishmeal is the main protein source to formulate aquafeeds which are largely derived from stocks of small pelagic fish. It is the basic ingredient for most fish diets because of its high protein content, balanced amino acid profile, high essential fatty acids content, minerals, and vitamins. However, the market price of fishmeal has risen significantly with the decrease in supply of stocks and increasing demand for aquaculture, as well as the high degradation of natural fish populations. Therefore, much work has been done to investigate alternative animal/plants protein sources, such as livestock by-products, and seafood processing by-products as substitutes for fishmeal in aquaculture feeds. The use of these ingredients in the diets of some carnivorous species has decreased the amount of fishmeal by approximately 35% [3]. Numerous studies have shown that animal by–product meals arising from the processing of slaughtered farm livestock offer great potential for use as dietary fishmeal replacements within aquaculture feed. Several animal protein sources have been evaluated to formuate the diets for different fish and shellfish species, such as poultry by-product meal [4,5], tuna muscle by-product powder [6], tuna liver meal [7] , fermented skipjack tuna viscera [8], tuna head hydrolyzates [9], brewers yeast [10] and soybean and brewer's grains [11]. It is important to know the response of spotted babylon to various nutrients in order to be able to maximize growth, improve body composition and produce an effective low-cost feeds for the species. Hence, this study was designed to determine the effects of partial to total replacement of fishmeal by poultry by-product meal in diets on growth performance and body composition of hatchery–reared juvenile spotted babylon (*Babylonia areolata*) under the flow-through system.

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Received January 06, 2015; **Accepted** February 05, 2015; **Published** March 15, 2015

Citation: Kritsanapuntu S, Chaitanawisuti N (2015) Replacement of Fishmeal by Poultry By-Product Meal in Formulated Diets for Growing Hatchery–Reared Juvenile Spotted Babylon (*Babylonia areolata*). J Aquac Res Development 6: 324. doi:10.4172/2155-9546.1000324

Materials and Methods

Experimental diets

The ingredients and formulation of the experimental diets are shown in Table 1. The protein sources fishmeal (54.68% protein), and poultry by-product meal (60.23% protein) were purchased from a commercial company. Poultry by-product meal was incorporated to replace fishmeal protein at 0%, 25%, 50%, 75% and 100% (diet PBM0, PBM25, PBM50, PBM75, and PBM100, respectively). Tuna oil served as the lipid source, and wheat flour was the carbohydrate source in the diets. Poultry by-product was ground to the desired particle size prior to preparing the diet. To prepare the diets, all dry ingredient poultry by-product meals were well mixed for 30 min in a food mixer. The tuna oil was then added, and mixed for 15 min. Finally, water (30% of dry weight ingredients) was added, and the medley mixed for 15 min. The diets were extruded and dried at room temperature for 48 h. For presentation, the feeds were shaped into small pieces (rounded discs 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 2012). Test diets (Table 1) contained similar levels of crude protein (40.82–41.49%) and crude fat content (17.49–17.93%).

Snail rearing and experimental design

B.areolata, juveniles (average weight, mean ± S.D, 0.10 ± 0.01 g) used in this experiment were obtained from a governmental hatchery (Rayong Coastal Fisheries Research and Development Center, Department of Fisheries, Rayong, Thailand). All juveniles came from the same production batch and were graded at the same size of 0.5 cm total shell length. The snails were allocated to 15 cylindrical plastic tanks (500 l/tank) with triplicate groups consisting of 300 snails each. Each tank was equipped with a flow-through system at a flow rate of 70 l/min. Juveniles were trained to accept formulated feed for 10 days prior to the experiment. The juveniles were hand fed once daily (10:00 h) to apparent visual satiation with the experimental diets. The amount of feed was adjusted daily based on the consumption by the snails within 0.5 h on the previous day to ensure that only a minimal quantity of feed remained. Apparent satiation was determined from observation when the snails ceased active feeding, and moved away from the feeding area and to bury themselves under the sand substratum. Uneaten food was siphoned out immediately after the snails stopped eating to prevent contamination of the water and sand substratum. The amount of feed eaten was recorded daily for calculation of the feed conversion ratio. All rearing tanks were provided with continuous aeration and maintained under a natural light/dark regime (12:12 h). Water temperature, pH, and salinity (mean ± S.D) were 28.2 ± 0.84°C, 8.1 ± 0.24, and 29.8 ± 0.49‰, respectively, during the experimental period. No chemicals or antibiotic agents were used throughout the entire experimental period. Grading by size was not carried out in any tank throughout the growing-out period. 80% of the snails in each tank were randomly sampling, and weighed individually every 30 days. Mortalities were recorded daily. The feeding trial was conducted for 150 days.

Ingredients (%)	PBM0	PBM25	PBM50	PBM75	PBM100
Fish meal	64.50	48.37	32.25	16.12	0
Poultry by-product meal	0	14.50	29.00	43.50	58.00
Soybean meal	5.0	5.0	5.0	5.0	5.0
Wheat flour	3.0	3.0	3.0	3.0	3.0
Wheat gluten	4.77	4.77	4.77	4.77	4.77
Tuna oil	11.54	11.54	11.54	11.54	11.54
Vitamin premix[a]	4.0	4.0	4.0	4.0	4.0
Mineral premix[b]	4.0	4.0	4.0	4.0	4.0
Cellulose	3.19	4.82	6.44	8.07	9.69
Proximate composition (g/100g dry sample)					
Crude protein	41.20	41.42	41.49	40.96	40.82
Crude fat	17.56	17.49	17.89	17.81	17.93
Ash	14.32	14.42	14.26	14.38	14.35
Moisture	12.22	12.27	12.25	12.28	12.26

Remarks:

[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}

PBM0=Fishmeal 100% and poultry meal 0%
PBM25=Fishmeal 75% and poultry meal 25%
PBM50=Fishmeal 50% and poultry meal 50%
PBM75=Fishmeal 25% and poultry meal 75%
PBM100=Fishmeal 0% and poultry meal 100%

Table 1: Ingredients and proximate composition on a dry weight basis of five experimental diets for hatchery–reared juvenile *B. areolata*.

Nutritional analysis

At the end of the 180 days growth trial, nutritional analysis was carried out on 200 randomly selected snails from each treatment to determine if experimental diets influenced the proximate composition, cholesterol, fatty acid profile and amino acid composition of *B. areolata*. The analysis of proximate composition included amounts of crude protein, crude lipid, ash and moisture of whole flesh of the experimental snails. Shells and opercula were removed for analysis of the whole wet flesh. Flesh from each replicate was combined and then split into three replicate samples and weighed for analysis. All samples were analyzed for proximate composition using the standard method of AOAC [12], cholesterol, fatty acid profile and amino acid composition by the private company, Central Laboratory (Thailand) Co. Ltd, Bangkok, Thailand as follows: Proximate composition of diets and whole flesh expressed on a dry matter basis was determined in triplicate according to standard procedures. The moisture content of each 2 g sample was calculated by drying to constant weight at 60°C for 24 h. Total nitrogen content was determined by the micro-kjeldahl method, and percentage crude protein was then calculated as %N×6.25. Total fat concentration was determined by Soxhlet extraction with petroleum ether as solvent carrier, and the crude fat was calculated gravimetrically. Ash content was determined by calcining samples to 550°C for 6 h. The amino acid (AA) composition (mg AA/100 g protein) of individual defatted diets in triplicate groups was determined. Samples (20 mg) were hydrolyzed with 200 µL of 6 N HCl and 0.06% phenol in a closed vial and heated to 110°C for 24 h. Amino acid profiles were determined following Waters AccQ-Tag procedure. Hydrolyzed samples were dried at 60°C with nitrogen and rehydrated with 1 mL water HPLC grade. Samples were filtered (0.45 µm) and derivatized using the Waters system AccQ-Tag. An HPLC Waters was used to chromatograph through a reverse phase column (3.9Å~150 mm) 4-µm Nova Pak C-18, using the water-acetonitrile gradient and a fluorescence detector (excitation and emission wave-length: 250 and 395 nm respectively). Analyses were conducted at a constant temperature of 39°C. Calibration and standard curves were obtained using an amino acid standard solution at three different concentrations (18.75-150 pmol of each amino acid) to calculate the amount of each AA, reported as percentage of each AA in the dry matter [13].

Data analysis

At the end of the experiment, the growth performance was assessed by determination of feed consumption (FC), weight gain (WG), absolute growth rate (AGR), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and survival rate as described by Hernandez et al. as follows:

Weight gain (g)=final mean weight (g)–initial mean weight (g)

Absolute growth rate (g/month)=[final mean weight (g)-initial mean weight (g)]/feeding trial period (month)

Specific growth rate (% day^{-1})=[ln final mean weight (g)–ln initial mean weight (g)/number of days] × 100

Feed conversion ratio (FCR)=Total feed intake (g)/weight gain (g)

Protein efficiency ratio (PER)=Total weight gain/total protein intake

Survival rate (%)=100×(Final snail number)/(initial snail number).

Statistical analysis

All data were presented as mean ± SD (n value as stated). The effects of dietary treatment on growth performance were analyzed by one-way analysis of variance (ANOVA) followed, where appropriate, by Tukey's post hoc test. The relationship between dietary treatment and chemical composition wa analyzed by regression analysis. ANOVA and regression analysis were performed using a SPSS statistical Software System version 14. Differences were regarded as significant when $P<0.05$.

Results

Growth performance

The results of overall growth performance, feed intake and final survival rate of juvenile spotted babylon *B. areolata* at the end of the 150 days feeding trials are shown in Figures 1-4. Significant differences ($P<0.05$) in weight gain, absolute growth rate, specific growth rate, feed conversion ratio and protein efficiency ratio were observed among the snails fed diets containing 0, 25, 50, 75, and 100% replacement of fishmeal by poultry by-product meal, except for final survival rate (Table 2). There were no significant differences ($P>0.05$) in specific growth rate among snails fed diets of PBM25, PBM50, and PBM75 which ranged from 2.19–2.21% day^{-1}, and these were significantly higher than the snails fed diets of PBM0 and PBM100 (2.03-2.12% day^{-1}, respectively). Final survival rates of snails ranged from 92.73%–93.94% and did not differ significantly ($P>0.05$) among the feeding treatments. There was significant difference ($P<0.05$) in total feed consumption where snails consumed diets of PBM0, PBM25, PBM50, and PBM75 (1,110-1,136 g), much more than the snails fed a diet of PBM100 (1,053 g). Significant differences ($P<0.05$) were found in feed conversion ratio

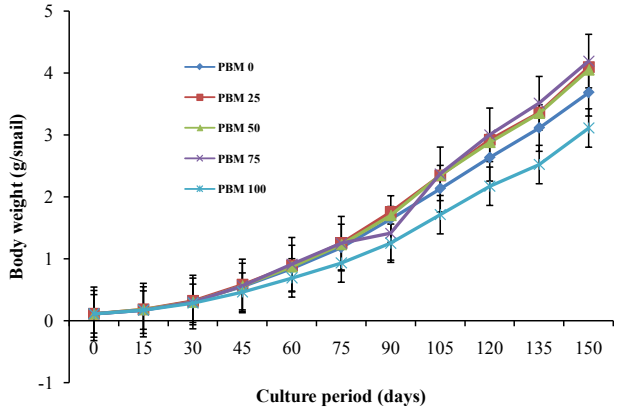

Figure 1: Average growth (g/snail) of hatchery–reared juvenile *B. areolata* fed different experimental diets for 150 days.

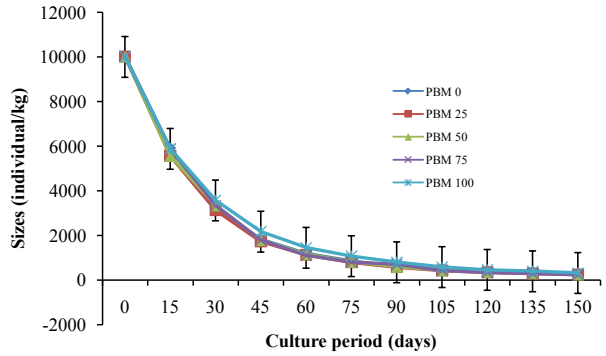

Figure 2: Average growth (individual/kg) of hatchery–reared juvenile *B. areolata* fed different experimental diets for 150 days.

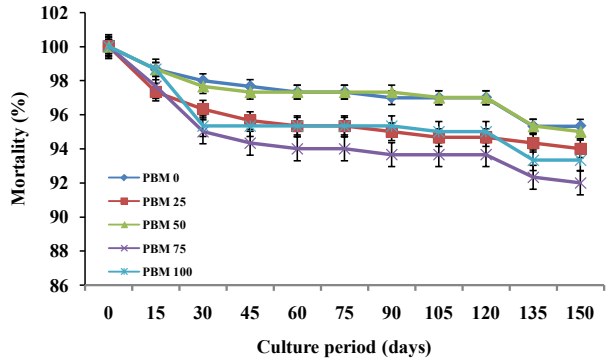

Figure 3: Cumulative percentage survival of hatchery–reared juvenile *B. areolata* fed different experimental diets for 150 days.

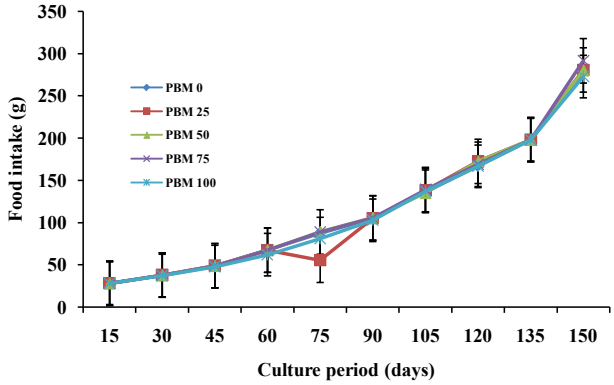

Figure 4: Average food intake of hatchery–reared juvenile *B. areolata* fed different experimental diets for 150 days.

Parameters	PBM0	PBM25	PBM50	PBM75	PBM100
Initial body weight (g/snail)	0.11 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.11 ± 0.01
Final body weight (g/snail)	3.68 ± 0.25	4.09 ± 0.18	4.05 ± 0.23	4.19 ± 0.07	3.11 ± 0.07
Initial sizes (indv. kg^{-1})	10000 ± 0.01	10000 ± 0.01	10000 ± 0.01	10000 ± 0.01	10000 ± 0.01
Final sizes (indv.kg^{-1})	271.7 ± 19.09^{b}	244.9 ± 10.91^{c}	246.9 ± 17.10^{c}	238.7 ± 1.58^{c}	321.5 ± 4.58^{a}
Body weight gain (g/snail)	3.58 ± 0.25^{c}	3.99 ± 0.18^{b}	3.98 ± 0.28^{b}	4.08 ± 0.12^{b}	3.04 ± 0.24^{d}
Absolute growth (g mo^{-1})	0.72 ± 0.05^{c}	0.79 ± 0.03^{b}	0.80 ± 0.06^{b}	0.82 ± 0.02^{b}	0.62 ± 0.01^{d}
Specific growth rate (% day^{-1})	2.12 ± 0.07^{c}	2.19 ± 0.03^{b}	2.19 ± 0.04^{b}	2.21 ± 0.01^{b}	2.03 ± 0.01^{d}
Total food intake (g)	1110 ± 5.81^{d}	1121 ± 8.04^{b}	1124 ± 13.13^{b}	1136 ± 8.45^{c}	1053 ± 10.08^{e}
Food conversion rate	1.18 ± 0.05^{b}	1.08 ± 0.08^{c}	1.02 ± 0.08^{c}	1.04 ± 0.03^{c}	1.81 ± 0.03^{a}
Protein efficiency ratio	0.51 ± 0.03^{b}	0.48 ± 0.07^{c}	0.45 ± 0.2^{c}	0.44 ± 0.01^{c}	0.59 ± 0.02^{a}
Final survival rate (%)	93.22 ± 2.37	93.67 ± 2.52	93.94 ± 0.47	93.28 ± 0.33	92.73 ± 0.37

Remarks:
PBM0=Fishmeal 100% and poultry meal 0%
PBM25=Fishmeal 75% and poultry meal 25%
PBM50=Fishmeal 50% and poultry meal 50%
PBM75=Fishmeal 25% and poultry meal 75%
PBM100=Fishmeal 0% and poultry meal 100%
Value within the same column followed by different letter superscripts were significantly different (P<0.05).
Values are means of three replicates per treatment.

Table 2: Growth performance of hatchery–reared juvenile *B. areolata* fed different experimental diets for 150 days. Values within the same row with different superscripts are significantly different (P<0.05).

Parameters	PBM 0	PBM 25	PBM 50	PBM 75	PBM 100
Protein (%N x 6.25)	17.02^{c}	17.41^{b}	19.32^{a}	16.81^{d}	15.29^{e}
Fat	4.76^{d}	4.68^{d}	6.29^{a}	4.83^{c}	5.05^{b}
Cholesterol	163.38^{a}	163.42^{a}	160.01^{b}	135.65^{c}	130.32^{d}
Carbohydrate	6.74^{b}	6.06^{c}	3.07^{e}	5.70^{d}	7.90^{a}
Ash	2.78^{a}	2.77^{a}	2.70^{a}	2.73^{a}	3.24^{b}
Moisture	68.97^{c}	69.08^{b}	68.62^{d}	69.93^{a}	68.52^{d}

Remarks:
PBM0 = Fishmeal 100% and poultry meal 0%
PBM25 = Fishmeal 75% and poultry meal 25%
PBM50 = Fishmeal 50% and poultry meal 50%
PBM75 = Fishmeal 25% and poultry meal 75%
PBM100 = Fishmeal 0% and poultry meal 100%

Table 3: Whole body composition (g/100g dry sample) of *B. areolata* fed different experimental diets for 150 days.

among the feeding treatments. Snails fed diets of PBM25, PBM50, and PBM75 displayed better feeding conversion ratios (1.02-1.08) and did not differ significantly (P>0.05) among one another, while the snails fed diets of PBM0 and PBM100 showed the poorer feed conversion ratios which ranged from 1.18-1.81. Protein efficiency ratios (PER) of snails fed diets of PBM100 (0.59) and PBM0 (0.51) were significantly higher (P<0.05) than other feeding treatments which ranged from 0.44-0.48.

Body composition of experimental snails

The proximate compositions of the whole body of juvenile spotted babylon *B. areolata* at the end of the 150 days feeding trials are shown in Table 3. Significant differences (P<0.05) were found in protein, fat, carbohydrate, ash and moisture levels among all feeding treatment groups. The snails fed diets of 50% replacement of fishmeal by poultry by–product meal (PBM50) resulted in the highest protein and fat contents compared with snails fed the diets of PBM0, PBM25, PBM75, and PBM100.

Cholesterol contents in the different treatment groups after the 150 days culture period are presented in Table 3. Significant difference (P<0.05) in cholesterol content was found among the feeding treatments. Cholesterol contents in snails fed diets of PBM75 (135.65 mg/100 g) and PBM100 (130.32 mg/100 g) were significantly lower (P<0.05) than those fed diets of PBM0 (163.38 mg/100 g), PBM25 (163.42 mg/100 g) and PBM50 (160.01 mg/100 g).

Amino acid	PBM0	PBM25	PBM50	PBM75	PBM100
Alanine	899.44	1017.07	905.85	1030.64	914.43
Proline	735.34	900.96	774.98	995.08	850.66
Serine	680.73	772.71	707.32	807.76	691.16
Aspartic acid	1334.39	1473.15	1331.75	1520.06	1294.32
Cystine	161.55	190.72	183.92	197.52	171.26
Glutamic acid	2194.41	2482.30	2185.44	2560.70	2212.02
Glycine	922.56	1189.86	948.00	1295.03	982.73
Tyrosine	416.57	495.64	421.91	511.40	494.03
Hydroxyproline	324.00	489.52	362.95	579.38	443.37
∑ Non-essential amino acid	**7668.99**	**9011.93**	**7822.12**	**9497.57**	**8053.98**
Arginine	916.42	1131.01	932.48	1195.34	967.68
Histidine	249.86	269.96	260.91	271.51	268.38
Isoleucine	260.22	489.52	257.07	353.67	324.66
Leucine	864.29	321.21	868.30	996.43	882.75
Lysine	703.29	983.09	657.44	714.63	631.38
Methionine	415.26	721.06	354.15	414.80	325.93
Phenylalanine	470.11	432.73	469.66	549.83	499.96
Threonine	520.53	619.14	525.29	657.16	581.92
Tryptophane	110.85	147.55	186.81	155.08	134.54
Valine	368.72	474.82	370.64	511.49	445.25
∑ Essential amino acid	**4879.55**	**5590.09**	**4882.75**	**5819.94**	**5062.45**

Remarks:
PBM0 = Fishmeal 100% and poultry meal 0%
PBM25 = Fishmeal 75% and poultry meal 25%
PBM50 = Fishmeal 50% and poultry meal 50%
PBM75 = Fishmeal 25% and poultry meal 75%
PBM100 = Fishmeal 0% and poultry meal 100%

Table 4: Amino acid compositions of whole body of *B. areolata* fed different experimental diets for 150 days (AA mg/100g dry sample).

Amino acid compositions in the different treatment groups after the 150 days culture period are presented in Table 4. There were 9 non-essential amino acids and 10 essential amino acids. Significant differences (P<0.05) in total non-essential amino acids and total essential amino acids were found among feeding treatments. The snails fed a diet of PBM75 showed the highest total non-essential amino acids (9,497.57 mg/100 g) and total essential amino acids (5,819.94 mg/100 g), while snails fed a diet of PBM0 showed the lowest total non-essential amino acids (7,668.99 mg/100 g) and total essential amino acids (4,879.55 mg/100 g).

Fatty acid	PBM0	PBM25	PBM50	PBM75	PBM100
C12:0	5.39	8.68	82.40	9.11	7.24
C13:0	2.31	20.6	2.41	1.56	1.65
C14:0	169.75	155.12	220.84	134.85	136.19
C15:0	41.94	36.55	43.24	26.43	25.66
C16:0	1048.59	1011.14	1325.99	988.39	1042.28
C17:0	67.12	59.78	69.95	48.80	48.52
C18:0	341.74	337.25	448.78	383.29	418.33
C20:0	21.10	20.98	26.05	21.30	21.22
C21:0	4.76	5.00	6.47	5.15	4.98
C22:0	14.76	15.12	18.79	17.90	18.26
C23:0	7.39	4.94	4.69	4.75	4.20
C24:0	26.41	28.63	32.59	20.73	18.24
∑ Saturated fatty acid	**1751.26**	**1685.25**	**2282.20**	**1662.26**	**1746.77**
C14:1	1.70	1.51	2.25	1.62	2.00
C16:1n7	237.09	211.51	256.84	164.03	165.84
C18:1n9t	36.89	38.57	50.91	36.88	36.35
C18:1n9c	725.60	798.26	1146.26	1006.18	1036.13
C20:1n11	86.04	87.54	118.48	104.20	124.07
C22:1n9	9.59	9.48	11.54	8.15	8.29
C24:1n9	18.30	17.33	30.04	17.73	16.56
∑ Monounsaturated fatty acid	**1115.21**	**1164.20**	**1616.3**	**1338.79**	**1389.24**
C18:2n6	457.67	492.19	724.49	678.52	700.06
C18:3n6	4.14	3.82	4.64	3.10	3.37
C18:3n3 linolenic acid	54.43	54.76	76.91	77.89	79.91
C20:2	28.21	29.02	35.47	30.91	37.50
C20:3n6	8.04	7.93	8.67	6.69	6.73
C20:3n3	8.22	7.89	8.46	7.25	7.26
C20:4n6	203.44	177.66	197.29	131.58	137.97
C20:5n3 EPA	252.23	229.64	274.31	184.05	201.39
C22:6n3 DPA	651.12	605.53	733.81	478.91	499.04
∑ n-6 PUFA	**673.29**	**681.60**	**935.09**	**819.89**	**848.13**
∑ n-3 PUFA	**966.00**	**897.82**	**1093.49**	**748.10**	**787.60**
∑ Polyunsaturated fatty acid	**1667.50**	**1608.35**	**2064.05**	**1598.90**	**1673.47**
∑ Unsaturated fatty acid	**2782.71**	**2772.55**	**3680.37**	**2937.69**	**3062.71**

Remarks:
PBM0=Fishmeal 100% and poultry meal 0%
PBM25=Fishmeal 75% and poultry meal 25%
PBM50=Fishmeal 50% and poultry meal 50%
PBM75=Fishmeal 25% and poultry meal 75%
PBM100=Fishmeal 0% and poultry meal 100%

Table 5: Fatty acid compositions of whole body of *B areolata* fed different experimental diets for 150 days (FA mg/100 g dry sample).

Fatty acid composition in the different treatment groups after the 150 days culture period are presented in Table 5. The whole body of snails fed PMB50 was significant higher (P<0.05) in saturated fatty acid, monounsaturated fatty acid, polyunsaturated fatty acid and unsaturated fatty acid contents than the groups of snails fed PBM0, PBM25, PBM100, and PBM75. There were significant differences (P<0.05) in eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), arachinodic acid (ARA), n-6 PUFA, and n-3 PUFA contents among the feeding treatments. The whole body of snails fed PMB50 contained the highest EPA, DHA, ARA, n-6 PUFA, and n-3 PUFA contents while those of snails fed PMB75 showed the lowest EPA, DHA, ARA, n-6 PUFA, and n-3 PUFA contents.

Discussion

This study provided useful information regarding the replacement of fishmeal in diets for juvenile *B. areolata* and the potential utilization of poultry by-product meal (PBM) as an alternative ingredient. PBM could potentially substitute up to 75% of fishmeal without reducing snail performance but increasing levels of amino acid and fatty acid composition. The use of PBM showed that snails fed diets of PBM25, PBM50, and PBM75 displayed better specific growth rates which ranged from 2.19-2.21% day^{-1} and did not differ significantly (P>0.05) while snails fed diets of PBM0 and PBM100 resulted poorer specific growth rates of 2.03-2.12% day^{-1}, respectively. The results showed that poultry by-product meal can effectively replace 50-75% fishmeal protein without a negative impacts on the biological indices for both growth and survival of *B. areolata* juveniles. Moreover, the inclusion of up to 75% poultry by-product meal in the diet improved feed efficiency and body composition. The snails fed diets of PBM25, PBM50, and PBM75 had significantly better growth rates than those of PBM0 and PBM100, but they were still lower than those of snails fed the conventional trash fish, formulated feeds [14,15] and supplementation with brewer's yeast [16]. This study showed that snails fed diets of PBM25, PBM50, and PBM75 had better growth rate than snails fed diet of 100% fishmeal inclusion. These findings were in agreement with the conclusions of previous studies indicating that PBM could be successfully applied at up to 25-50% for rose snapper [17], 20% for common carp, and 44% for rainbow trout. In addition, Hernandez et al. [17] indicated that PBM meal may have differing constituents (e.g., bone, meat and blood), nutrient compositions, processing methods and digestibility. If high–quality PBM is used, many species tolerate replacement levels up to 100%. Likewise, Hernandez etal. indicated that replacing 25% of fish meal protein by poultry by-product meal showed a similar trend for feed efficiency and growth performance in juvenile spotted rose snapper (*Lutjanus guttatus*) than the control diet. However, growth performance was reduced at 75% level of fish meal protein replacement by poultry by-product meal, dueto deficiencies of lysine and methionine. Nasution and Roberts [18] showed that juvenile common whelks, *Buccinum undatum,* fed on blue mussels had the highest survival rate, followed by those fed on a combination of other experimental diets, cod waste and fish-feed pellets. In addition, this study indicated that the body composition of snails was affected by the replacement of fish meal with PBM meals. Sierra et al. [13] found that juvenile rainbow trout (*Oncorrhynchus mykiss*) fed fishmeal from tuna fish by-products and poultry by-product meal (PBM) showed no significant differences in terms of thermal unit growth coefficient. They concluded that PBM could be used up to 44% in diets for juvenile rainbow trout without a significant decrease in EPA and DHA. The use of a whole fishmeal diet could be an important strategy to recuperate the fatty acid profile obtained when trout is fed on PBM basis. It will be important to perform longer experiments with larger fish to confirm these results. In conclusion, the results of this first study showed that for *B. areolata* juveniles, up to 75% of the fishmeal protein in formulated diets can be replaced by poultry by-product meal. It is also clear that *B. areolata* can accept PBM as a fishmeal alternative protein without any negative effects on health and growth performance. Based on the economic performance of the spotted babylon fed with the experimental diets, the replacement of fishmeal with poultry by-product meal is recommended. This study presents the first research conducted on the nutritional capacity of the spotted babylon and may serve as a basis for future studies.

Acknowledgment

This research was funded by the research budget of Prince of Songkla University in fiscal year 2014. I would like to thank the Faculty of Science and Industrial Technolgy, Prince of Songkla University, Surattani Campus for supporting scientific instruments and facilities as well as other positive encouragements while the research was being conducted.

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Citation: Kritsanapuntu S, Chaitanawisuti N (2015) Replacement of Fishmeal by Poultry By-Product Meal in Formulated Diets for Growing Hatchery–Reared Juvenile Spotted Babylon (*Babylonia areolata*). J Aquac Res Development 6: 324. doi:10.4172/2155-9546.1000324