Use of Tuna-Cooking Liquid Effluent as a Dietary Protein and Lipid Source Replacing Fishmeal in Formulated Diets for Growing Hatchery-Reared Juvenile Spotted Babylon (*Babylonia areolata*)

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Abstract

This study presented the first research conducted on the use of tuna by-product from the tuna canning industry for growing hatchery-reared juvenile spotted babylon (*Babylonia areolata*) to marketable sizes. A feeding trial was conducted to evaluate the effects of five levels of partial to complete replacement of fishmeal by tuna-cooking liquid effluent on growth performance and body composition of snails reared under a flow-through culture system over 150 days. Five experimental diets were formulated to contain 0%, 25%, 50%, 75%, and 100% of tuna-cooking liquid effluent (diets TCLE0, TCLE25, TCLE50, TCLE75, and TCLE100, respectively). Results showed that significant differences (P<0.05) in specific growth rate, feeding conversion ratio, and protein efficiency ratio were observed among the snails fed diets containing 0, 25, 50, 75, and 100% replacement of fishmeal by tuna-cooking liquid effluent meal. The best specific growth rate, feeding conversion ratio, and protein efficiency ratio were found in the group of snails fed a diet of TCLE100, while the lowest specific growth rate, feeding conversion ratio, and protein efficiency ratio were found in snails fed diets of TCLE0 and TCLE25. No significant differences (P>0.05) in final survival rate was found among snails fed all experimental diets. Survival rates ranged from 94.2%-94.6%. Moreover, the snails fed diets of 100% replacement of fishmeal by tuna-cooking liquid effluent meal (TCLE100) showed the highest protein content, lowest lipid content, and lowest cholesterol content compared with snails fed all the other diets. The whole body composition of snails fed TCLE50 was significantly higher (P<0.05) in saturated fatty acid, monounsaturated fatty acid, polyunsaturated fatty acid, unsaturated fatty acid, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), arachidonic acid (ARA), n-6 PUFA, and n-3 PUFA contents than the groups of snails fed all the other diets. The results of this study indicated that tuna-cooking liquid effluent meal can completely replace fishmeal protein with positive effects on snail growth performance and whole body composition.

Keywords: *Babylonia areolata*; Fishmeal; Tuna-cooking liquid effluent; Growth performance; Body composition

Introduction

Spotted babylon, *Babylonia areolata*, are generally carnivores and feed mostly on the fresh meat of trash fish. However, feeding fish meat to spotted babylon snails entails problems including variability in nutritive content and supply, thus resulting in a slow and heterogeneous growth rate of the species. As a result of these issues intensive spotted babylon culture is becoming increasingly reliant upon formulated practical diets. 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 their production in Thailand [1-3]. In addition, [4] indicated that juvenile Dog conch (*Strombus canarium*), fed a formulated diet with 38.50% protein content had the highest growth performance with no significant differences in survival rate and food conversion ratio compared to diets containing 56.68% and 37.10% protein content. However, fishmeal is the main protein source to formulate aquafeeds which are largely derived from stocks of small pelagic fish. This is the basic ingredient for most fish diets because of its high protein content with a balanced amino acid profile; it is a good source of essential fatty acids, minerals, and vitamins. However, the market price of fishmeal has risen significantly, due to the decrease in supply of stocks with the high degradation of natural fish populations and increasing demand for aquaculture. Therefore, at lot of work has been done to investigate alternative animal/plant protein sources, such as livestock and seafood processing by-products to substitute for fishmeal in aquaculture feeds.

The use of such ingredients in the diets of some carnivorous species has decreased the amounts of fishmeal used by 35% [5].

The main structural factors that determine the profitability of the tuna-canning sector is the low performance of the production process, which results in losses of 50%. The losses are particularly high during the cutting, cooking, and peeling stages. 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 replacers within aquaculture feed. Several tuna waste products used as animal protein sources were evaluated to formulate the diets for different fish and shellfish species, such as tuna muscle by-product powder [6], tuna fishmeal for rainbow trout [5], tuna liver meal for common carp [7], fermented skipjack tuna viscera for abalone [8], tuna head hydrates for white shrimp [9], co-extruded tuna viscera for white shrimp [10], and tuna silage hydrates for Nile tilapia [11]. One possibility of improving performance in the tuna-canning sector is the recycling of...
the wastes obtained before packing, particularly protein recovery from the steam cooking effluents. Tuna cooking water is brine resulting from the cooking process; it contains pieces of fish meat, sarcoplasmic proteins, and a small proportion of solubilized myofibrillar proteins. In greater proportion the brine also contains gelatin, resulting from the fusion of collagen during cooking. This residue therefore presents a high organic load and a strong contamination impact. For this study, desalination and recovery of the collagenous fraction from the tuna cooking water was evaluated [12]. It is important to learn 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 feed for the species. Hence, this study was designed to determine the effects of partial to total replacement of fishmeal by tuna-cooking liquid effluent as a dietary protein and lipid source in the diet on growth performance and body composition of hatchery-reared juvenile spotted babylon (Babylonia areolata) under the flow-through system.

Materials and Methods

Experimental diets

The ingredients and formulation of the experimental diets is shown in Table 1. The protein source, tuna-cooking liquid effluent (60.23%) was obtained from a fish canning company, Kuang Pei San Food Products Public Co., Ltd., Trang Province. Tuna-cooking liquid effluent was incorporated to replace poultry by-product protein at 0%, 25%, 50%, 75%, and 100% (diets TCLE0, TCLE25, TCLE50, TCLE75, and TCLE100, respectively). Tuna oil served as the lipid source and wheat flour was the carbohydrate source in the diets. The poultry by-product was ground to the desired particle size prior to preparing the diets. To prepare 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 was again mixed for 15 min. The diets were extruded and dried at room temperature for 48 h. For feeding, the mixture was formed into small pieces (round discs of 1.5 cm diameter) to facilitate sucking by the snails. All experimental diets were then stored in a refrigerator at -20°C until use. All diets were analyzed in triplicate for the proximate compositions according to standard methods (AOAC 2012). Test diets (Table 1) contained similar levels of crude protein (40.62-43.88%) and crude fat content (8.94-16.39%).

Snail rearing and experimental design

B. areolata, juveniles (average weight, mean ± SE, 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 were from the same batch of production and graded at the same size of 0.5 cm total shell length. They 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 amount of food consumed by the snails within 0.5 h on the previous day to ensure that only a minimal amount of feed remained. Apparent satiation was determined when the snails ceased active feeding, moved away from the feeding area and buried themselves under the sand substrate. 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 natural light/dark regime (12:12 h). Water temperature, pH, and salinity (mean ± SE) were 28.2 ± 0.84°C, 8.1 ± 0.24, and 29.8 ± 0.49%, respectively. No chemicals or antibiotic agents were used throughout the entire experimental period. Grading by size was not carried out in any tank during the growing-out period. 80% of the snails in each tank were randomly sampled, and weighed individually every 30 days. Mortalities were recorded daily. The feeding trial was conducted for 150 days.

Nutritional analysis

At the end of the 150 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, and fatty acid composition of B. areolata. The analysis of proximate composition based on the standard methods of AOAC [13], included amounts of crude protein, crude fat, ash, and moisture of the whole flesh of the experimental snails. Shells and opercula were removed for analysis of the whole wet flesh composition. Flesh from each replicate was combined and then split into three replicate samples and weighed for analysis. All samples were analyzed for proximate composition, cholesterol, and fatty 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 samples according to standard procedures. The moisture content of each sample was calculated from 2 g samples dried 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 x 6.25. Total fat concentration was determined by Soxhlet extraction using petroleum ether as the solvent carrier; the crude fat was calculated gravimetrically. Ash content was determined by calcining samples at 550°C for 6 h.

Data analysis

At the end of the experiment, the growth performance was assessed by the 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 x 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 was 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

Growth in body weight of juvenile spotted babylon B. areolata fed experimental diets over a period of 150 days are shown in Figure 1. 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 tuna-cooking liquid effluent meal as shown in Table 2. The highest specific growth rate was found in snails fed a diet of TCLE100 (2.53% day−1) and the lowest specific growth rates were found in snails fed diets TCLE0 (2.16% day−1) and TCLE25 (2.19% day−1). No significant differences (P>0.05) in final survival were found among snails fed all the experimental diets, ranging from 94.2%–94.6%. Significant differences (P<0.05) were found in feed conversion ratio (FCR) and protein efficiency ratio (PER) among the feeding treatments. The best feeding conversion ratio was found in snails fed diets of TCLE100 (0.99) and TCLE75 (0.87), while the snails fed diets of TCLE0, TCLE25, and TCLE50 showed poorer feed conversion ratios ranging from 1.17–1.34. The best protein efficiency ratio was found in snails fed diets TCLE100 (2.71) and TCLE75 (2.35), while the snails fed diets TCLE0, TCLE25, and TCLE50 showed poorer feed conversion ratios ranging from 1.65–1.89.

Body composition of experimental snails

The proximate compositions and cholesterol content 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 a diet of 100% replacement of fishmeal by tuna-cooking liquid effluent meal (TCLE100) showed the highest protein content (18.68 g/100 g) and lowest fat content (1.31 g/100 g) compared with snails fed the other diets. Significant difference (P<0.05) in cholesterol content was found among the feeding treatments. Cholesterol contents in snails fed a diet TCLE100 (95.72 mg/100 g) was significantly lower than those fed diets of TCLE0 (128.47 mg/100 g), TCLE25 (111.22 mg/100 g), TCLE50 (113.50 mg/100 g) and TCLE75 (112.42 mg/100 g). Fatty acid composition in the different treatment groups after the 150 days culture period is presented in Table 4. The whole body composition of snails fed TCLE50 was significantly 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 the other diets. There were also significant differences (P<0.05) in eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), arachidonic acid (ARA), n-6 PUFA, and n-3 PUFA contents among the feeding treatments. The whole body of snails fed TCLE50 contained the highest EPA, DHA, ARA, n-6 PUFA, and n-3 PUFA contents.

Discussion

This study presents the first research conducted on the use of tuna by-product from the tuna canning industry for growing hatchery-reared juvenile spotted babylon snails (Babylonia areolata) to marketable sizes and may serve as a basis for future research work. Results indicated that there were significant differences in weight gain, absolute growth rate, specific growth rate, feed conversion ratio, and protein efficiency ratio among the snails fed diets containing 0, 25, 50, 75, and 100% replacement of fishmeal by tuna-cooking liquid effluent meal. Final survival rates were unaffected. The highest specific growth rate was found in snails fed a diet of TCLE100 and the lowest in snails fed diets of TCLE0 and TCLE25. The best feeding conversion ratio and protein efficiency ratio were found in snails fed diets of TCLE100 and TCLE75, while the snails fed diets of TCLE0, TCLE25, and TCLE50 showed poorer feed conversion ratio and protein efficiency ratio. The results showed that tuna-cooking liquid effluent meal can effectively replace 75–100% fishmeal protein without negative impacts on the biological indices for both growth and survival of B. areolata juveniles. Gumus et al. indicated that up to 20% of fishmeal protein in common carp fry (Cyprinus caprio) diet can be replaced by tuna liver meal (TLM) without adverse effects on growth, feed utilization,
showed that tuna by-products (tuna fishmeal and tuna fish oil) with (>35 mg/g) content than their bivalve prey. Iranshahi and Kiaalvandi (1.9 kcal/g), with significantly higher protein (>26 mg/g) and glycogen other gastropods in the culture. Their flesh had a high caloric value growth (up to 0.8 g/month) and higher survival (>90%) compared to required to subdue prey. Overall, juvenile D. orbita could reflect an optimal foraging strategy to minimize the energy and ash content. Dicathais orbita showed a preference in captivity and produced whelk flesh with significantly higher calorific energy but higher protein, glycogen, and lipid content than bivalve feeds results were in agreement with the conclusions of previous studies and effect on the zootechnical performance of the formulated diets. These They concluded that fraction separation after hydrolysis had a positive improved growth performances of shrimp (Litopenaeus vannamei). Insoluble protein powder from the hydrolysis of tuna head, significantly supplemented with soluble protein powders, as well as one containing and body composition. Likewise, Nguyen et al. [9] showed that diets supplemented with soluble protein powders, as well as one containing insoluble protein powder from the hydrolysis of tuna head, significantly improved growth performances of shrimp (Litopenaeus vannamei). They concluded that fraction separation after hydrolysis had a positive effect on the zootechnical performance of the formulated diets. These results were in agreement with the conclusions of previous studies and indicated that various types of tuna by-products gave good results in growth performance on fish and shellfish. Woodcock and Benkendorff [14] demonstrated that artificial pellets had significantly less moisture, but higher protein, glycogen, and lipid content than bivalve feeds and produced whelk flesh with significantly higher calorific energy and ash content. Dictaｈis orbita showed a preference in captivity for scavenging frozen bivalves, over predation on live mollusks. This could reflect an optimal foraging strategy to minimize the energy required to subdue prey. Overall, juvenile D. orbita displayed similar growth (up to 0.8 g/month) and higher survival (>90%) compared to other gastropods in the culture. Their flesh had a high caloric value (1.9 kcal/g), with significantly higher protein (>26 mg/g) and glycogen (>35 mg/g) content than their bivalve prey. Iranshahi and Kiaalvandi showed that tuna by-products (tuna fishmeal and tuna fish oil) with

| Parameters                      | TCLE0 | TCLE25 | TCLE50 | TCLE75 | TCLE100 |
|---------------------------------|-------|--------|--------|--------|---------|
| 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)     | 2.84 ± 0.06 | 3.00 ± 0.08 | 3.31 ± 0.07 | 4.19 ± 0.11 | 4.91 ± 0.09 |
| Body weight gain (g/snail)      | 2.74 ± 0.06a | 2.90 ± 0.08b | 3.21 ± 0.07a | 4.09 ± 0.05c | 4.81 ± 0.09b |
| Absolute growth (g mo⁻¹)        | 0.55 ± 0.01a | 0.58 ± 0.01a | 0.64 ± 0.01a | 0.82 ± 0.01c | 0.96 ± 0.02b |
| Specific growth rate (% day⁻¹)  | 2.16 ± 0.01a | 2.19 ± 0.01a | 2.27 ± 0.01a | 2.42 ± 0.01a | 2.53 ± 0.01a |
| Total food intake (g)           | 1110 ± 5.81 | 1121 ± 8.04 | 1124 ± 13.13 | 1156 ± 8.45 | 1187 ± 10.08 |
| Food conversion rate            | 1.34 ± 0.05a | 1.22 ± 0.04a | 1.17 ± 0.02b | 0.87 ± 0.03c | 0.99 ± 0.02 |
| Protein efficiency ratio        | 1.65 ± 0.07a | 1.66 ± 0.02a | 1.69 ± 0.08b | 2.35 ± 0.06b | 2.71 ± 0.07b |
| Final survival rate (%)          | 94.2 ± 1.63 | 94.2 ± 1.19 | 94.3 ± 2.44 | 94.6 ± 1.23 | 94.4 ± 1.01 |

Remarks:
TCLE0=Fishmeal 100% and tuna-cooking liquid effluent 0%
TCLE25=Fishmeal 75% and tuna-cooking liquid effluent 25%
TCLE50=Fishmeal 50% and tuna-cooking liquid effluent 50%
TCLE75=Fishmeal 25% and tuna-cooking liquid effluent 75%
TCLE100=Fishmeal 0% and tuna-cooking liquid effluent 100%

Value within the same column followed by different letter superscripts were significantly different (P<0.05).

Table 2: Growth performance of hatchery-reared juvenile B. areolata fed experimental diets containing 5 levels of tuna-cooking liquid effluent for 150 days. Values within the same row with different superscripts are significantly different (P<0.05)

| Parameters | TCLE0 | TCLE25 | TCLE50 | TCLE75 | TCLE100 |
|------------|-------|--------|--------|--------|---------|
| Protein (%N x 6.25) | 16.14 | 15.60 | 14.64 | 17.95 | 18.68 |
| Fat        | 1.92 | 1.61 | 2.14 | 1.59 | 1.31 |
| Cholesterol| 128.47 | 111.22 | 113.50 | 112.42 | 95.72 |
| Carbohydrate| 3.29 | 2.39 | 2.05 | 2.11 | 1.58 |
| Ash        | 4.77 | 5.38 | 4.16 | 5.40 | 4.77 |
| Moisture   | 73.88 | 75.02 | 77.01 | 72.95 | 73.66 |

Remarks:
TCLE0=Fishmeal 100% and tuna-cooking liquid effluent 0%
TCLE25=Fishmeal 75% and tuna-cooking liquid effluent 25%
TCLE50=Fishmeal 50% and tuna-cooking liquid effluent 50%
TCLE75=Fishmeal 25% and tuna-cooking liquid effluent 75%
TCLE100=Fishmeal 0% and tuna-cooking liquid effluent 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 3: Whole body composition (g/100 g dry sample) of B. areolata fed experimental diets containing 5 levels of tuna-cooking liquid effluent for 150 days.

| Parameters | TCLE0 | TCLE25 | TCLE50 | TCLE75 | TCLE100 |
|------------|-------|--------|--------|--------|---------|
| Protein    | 16.14 | 15.60 | 14.64 | 17.95 | 18.68 |
| Fat        | 1.92 | 1.61 | 2.14 | 1.59 | 1.31 |
| Cholesterol| 128.47 | 111.22 | 113.50 | 112.42 | 95.72 |
| Carbohydrate| 3.29 | 2.39 | 2.05 | 2.11 | 1.58 |
| Ash        | 4.77 | 5.38 | 4.16 | 5.40 | 4.77 |
| Moisture   | 73.88 | 75.02 | 77.01 | 72.95 | 73.66 |

In the case of fatty acid composition of whole body of B. areolata, the results showed that the percentage of the total monounsaturated fatty acid was significantly higher than the other levels. The total n-3 PUFA content was also significantly higher in the TCLE0 diet, while the total n-6 PUFA content was higher in the TCLE100 diet. This suggests that the substitution of fishmeal with tuna-cooking liquid effluent may be beneficial for B. areolata in terms of fatty acid composition, especially for the ω-3 fatty acids necessary for optimal health and growth. Overall, the findings of this study indicate that the use of tuna-cooking liquid effluent as a dietary protein and lipid source can be an effective strategy to reduce the dependence on fishmeal, while still providing adequate nutrition for B. areolata.
the present quality were not qualified protein or energy sources for rainbow trout (Oncorhynchus mykiss) at high inclusion levels. However, further studies are required to investigate the processing techniques and storage conditions of these products to resolve the palatability problem. Hernandez et al. stated that Nile tilapia (Oreochromis niloticus) fed a tuna by-product meal (TBM) diet had greater weight gain and feed intake, and lower feed conversion ratios than those fed diets containing tuna silage hydrolysis (TSH). However, fish fed diets T75%, T90%, and T100% showed reduced growth performance. Moreover, Hernandez et al. [10] found that co-extracted wet tuna viscera can make up to 40% of the practical diets of shrimp (Litopenaeus vannamei) without any detrimental effects. Lee Kim and Kim indicated that fermented skipjack tuna viscera can be used as a partial substitute protein source for fishmeal or soybean meal in the formulated diet for juvenile abalone. Their results indicated that snails fed diets of 100% replacement of fishmeal by tuna-cooking liquid effluent meal (TCE100) showed the highest protein content, lowest fat content, and lowest cholesterol content compared to snails fed diets of TCE0%, TCE25%, TCE50%, and TCE75% [15]. However, snails fed TCE50 showed the best results for saturated fatty acid, monounsaturated fatty acid, polyunsaturated fatty acid, unsaturated fatty acid, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), arachidonic acid (ARA), n-6 PUFA, and n-3 PUFA content than snails fed the other diets. For improvement of body composition, results of this study indicated that tuna-cooking liquid effluent meal can completely replace fishmeal protein with positive effects in snail performance. A 50% replacement can improve body composition particularly EPA, DHA, ARA, n-6 PUFA, and n-3 PUFA contents. It is also clear that B. areolata prefers TCE100% 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 tuna by-product from the tuna canning industry meal is recommended. Further studies are required to evaluate other by-products from the tuna canning industry for the partial or complete replacement of fishmeal in B. areolata diets. More studies are needed to determine the economic viability of the large-scale use of these components in snail feed formulation.

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