Fish hydrolysate supplemented diet improved feed efficiency and growth of coral trout (*Plectropomus leopardus*)

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Abstract. Abstract. Fish hydrolysate contains hydrolyzed protein which comprised of peptides and free amino acids that are easily digested and absorbed by fish. This study aimed to determine the effectiveness of fish hydrolysate in improving feed efficiency and growth of coral trout. Five experimental diets were formulated to contain 47% protein and 12% lipid with different sources and levels of fish hydrolysate. Diet-1 and diet-2 were added with crustacean hydrolysate at 2% and 4% levels, respectively. Whereas diet-3 and diet-4 were supplemented with 2% and 4% of tuna hydrolysate. Diet-5 was formulated without fish hydrolysate supplementation (control). The diets were prepared as dry pellet with 3 mm diameter. Coral trout juveniles were produced in the hatchery of Institute for Mariculture Research and Fisheries Extension (IMRAFE), Gondol-Bali with the average body weight of 7.6 ± 0.9 g. The juveniles were maintained in 15 fiber tanks, 400L in volume, with a density of 40 fish/tank. The experiment was designed with a completely randomized design (CRD) with 5 treatments (different diets) and 3 replications for each treatment. The fish were fed the experimental diets three times every day at satiation level for 84 days of feeding experiment. Results of the experiment showed that increased supplementation of fish hydrolysate from 2% to 4% in diet tended to improve fish growth performance. However, the best growth performance, in term of weight gain (WG) and specific growth rate (SGR), was obtained in fish fed diet supplemented with 4% crustacean hydrolysate. This growth performance was significantly higher than the other treatments (P<0.05). The best feed efficiency (FE) and protein efficiency ratio (PER) were also obtained in fish fed diet supplemented with 4% crustacean hydrolysate. The results of the present study showed that supplementation of 4% crustacean hydrolysate in diet effectively improved the growth and feed efficiency of coral trout.

1. Introduction

Coral trout grouper (*Plectropomus leopardus*), is an economically high fishery commodity with high price. However, to date, most of coral trout production still relies on the capture fisheries which is feared to threaten its natural population. Aquaculture business of coral trout needs to be developed promptly to anticipate the decline in wild stocks. The availability of fingerlings and aquafeed is pivotal to support the success in the development of aquaculture. Grouper is a carnivorous fish, and therefore, the protein requirement is relatively high. This requirement was reported up to 47.8 - 60.0%, which varies according to species [1]. Grouper is also known to be lack capability to utilize fat from diet as a source of energy [2]. Coral trout juvenile has been reported to require diet with a protein content of 47% for good growth [3]. Feeding behavior of coral trout is relatively slow when compared to other
groupers that have been widely cultivated, such as tiger grouper and hybrid grouper. Therefore, it is necessary to formulate diet that can increase feed consumption and efficiently support the growth of coral trout.

Fish hydrolysate is produced from by-products of fish processing industries. Fish hydrolysate generally contains high nutritional value and several free amino acids that are potential to be utilized as feed ingredients. Fish hydrolysate contains hydrolyzed protein which consists of peptides and free amino acids that are easier to digest and absorbed by fish. As a result, it increases fish appetite, growth and immunity [4, 5]. Fish protein hydrolysate also provide antioxidant and several functional properties that promote fish growth and health [6]. Nowadays, shrimp hydrolysate that is produced enzymatically, is widely used as a supplement in shrimp feed. Growth performance and immune response of Asian sea bass *Lates calcarifer* was also reported to increase when was fed fish hydrolysate supplemented diet, both in the nursery and grow-out phases [7].

To date, there is a lack of information on fish hydrolysate supplementation in diet and its response to the growth of coral trout. Taking into account the feeding behavior of coral trout and the characteristics of fish hydrolysate, this study aimed to determine the effectiveness of fish hydrolysate in increasing feed efficiency and growth of coral trout juvenile.

2. Material and methods

2.1. Coral trout juvenile

The coral trout juvenile were obtained from the hatchery of Institute for Mariculture Research and Fisheries Extension (IMRAFE), Gondol. Before being used in the feeding experiment, the juvenile were kept in a 2 x 2 x 0.5 m fiber tank with a flow-through water system. At the beginning of this adaptation phase, the juvenile were fed commercial artificial diet and then gradually accustomed to the diet made in the IMRAFE’s Feed Laboratory until they were ready for the experiment.

2.2. Experimental diets

The experimental diets were formulated to contain 47% protein and 12% lipid with the main protein sources from fish meal, soybean meal, mysid meal and squid liver meal. In the feed formulation, 2 types of hydrolysate were added, namely shrimp hydrolysate and tuna hydrolysate, each at 2% and 4% level, and one experimental diet was made without supplementation (control) (table 1). The diets were pelleted using a machine (Royal, Japan) with a diameter of 2 mm. The diets were dried in an oven at a temperature of 70 °C, and then stored in a refrigerator before and during the feeding experiment.

2.3. Feeding experiment

The experiment was held in the wet laboratory of IMRAFE from Februari to May 2020. This experiment used 15 fiber tank, 400 L in volume, in a flow-through water system (6 L / min.), equipped with aeration as oxygen supply. Prior to be flowed to the experimental tanks, sea water was filtered through a sand filter system and deposited in a reservoir.

Coral trout juvenile that had been adapted to the formulated diet and the experimental environment were selected, and measured individually for their length and weight. Juveniles with a total length of 8.3 ± 0.3 cm and a weight of 7.6 ± 0.9 g were allocated into the experimental tanks with a density of 40 individuals for each tank. The experiment was designed with a completely randomized design (CRD) with 5 experimental diet treatments and 3 replications for each treatment. The juveniles were fed the experimental diets in accord with the treatments 3 times a day at the satiation level for 84 days. The uneaten feed was collected, stored in a freezer and then dried to determine feed consumption. Measurement of fish length and weight was carried out every 14 days by measuring all fish in each tank. At the end of the experiment, 8 fish from each tank were taken and dried using a freeze dryer for analysis of the fish body chemical composition.
Table 1. Ingredient and proximate composition of experimental diets (% diet)

| Ingredients          | Control | Shrimp hydrolysate-2 | Shrimp hydrolysate-4 | Tuna hydrolysate-2 | Tuna hydrolysate-4 |
|----------------------|---------|----------------------|----------------------|--------------------|--------------------|
| Fish meal            | 50      | 48                   | 46                   | 48                 | 46                 |
| Fish hydrolysate     | 0       | 2                    | 4                    | 2                  | 4                  |
| Squid liver meal     | 12      | 12                   | 12                   | 12                 | 12                 |
| Mysid meal           | 10      | 10                   | 10                   | 10                 | 10                 |
| Soybean meal         | 10      | 10                   | 10                   | 10                 | 10                 |
| Wheat flour          | 9.5     | 9.5                  | 9.5                  | 9.5                | 9.5                |
| Fish oil             | 3       | 3                    | 3                    | 3                  | 3                  |
| Vitamin Mix          | 1.5     | 1.5                  | 1.5                  | 1.5                | 1.5                |
| Mineral Mix          | 1.5     | 1.5                  | 1.5                  | 1.5                | 1.5                |
| Astaxanthin          | 0.5     | 0.5                  | 0.5                  | 0.5                | 0.5                |
| Taurine              | 0.5     | 0.5                  | 0.5                  | 0.5                | 0.5                |
| CMC                  | 1.5     | 1.5                  | 1.5                  | 1.5                | 1.5                |
| Total                | 100     | 100                  | 100                  | 100                | 100                |

Proximate composition of the diets (% diet)

| Component            | Control | Shrimp hydrolysate-2 | Shrimp hydrolysate-4 | Tuna hydrolysate-2 | Tuna hydrolysate-4 |
|----------------------|---------|----------------------|----------------------|--------------------|--------------------|
| Dry matter           | 90.7    | 90.2                 | 90.8                 | 89.6               | 89.3               |
| Protein              | 46.8    | 47.2                 | 47.7                 | 46.9               | 46.6               |
| Lipid                | 12.1    | 12.8                 | 12.9                 | 12.5               | 12.5               |
| Ash                  | 13.7    | 13.6                 | 13.8                 | 13.6               | 13.5               |
| Digestibility of dry matter (%) | 73.1     | 73.0                 | 73.3                 | 71.1               | 71.9               |
| Digestibility of protein (%) | 91.2     | 91.2                 | 91.0                 | 90.2               | 90.2               |

2.4. The observed parameters and data analysis

The observed parameters included survival (SR), final weight (FW), weight gain (WG), specific growth rate (SGR), feed efficiency (FE), protein efficiency ratio (PER), protein retention (RP), lipid retention (LR), and the chemical composition of the fish body.

\[
\text{Survival Rate} (\%) = 100 \times \frac{N_t}{N_0} \tag{1}
\]

\[
\text{Weight Gain} (\%) = 100 \times \frac{(W_t - W_0)}{W_0} \tag{2}
\]

\[
\text{Specific Growth Rate} (\% \text{ day}^{-1}) = 100 \times \frac{(\ln W_t - \ln W_0)}{t} \tag{3}
\]

\[
\text{Feed intake (g)}
\]

\[
\text{DFC} (\% \text{day}) = \frac{\text{feed intake (g)}}{2} \tag{4}
\]

\[
\text{FE} = \frac{\text{Weight gain (g)}}{\text{feed intake (g)}} \tag{5}
\]

\[
\text{PER} = \frac{\text{Weight gain (g)}}{\text{protein intake (g)}} \tag{6}
\]
Final body protein content (g) – Initial body protein content (g)

\[ PR (\%) = \frac{Final \ body \ protein \ content \ (g) - Initial \ body \ protein \ content \ (g)}{Protein \ intake \ (g)} \times 100 \quad (7) \]

Final body lipid content (g) – Initial body lipid content (g)

\[ LR (\%) = \frac{Final \ body \ lipid \ content \ (g) - Initial \ body \ lipid \ content \ (g)}{Lipid \ intake \ (g)} \times 100 \quad (8) \]

Where \( N_t \) and \( N_0 \) are the number of fish at the end and the beginning of the experiment, \( W_t \) and \( W_0 \) are the average fish weights at the end and the beginning of the experiment, and \( t \) is the length of the experiment (days).

To evaluate the response of coral trout juvenile to the experimental diets, data of survival, weight gain, specific growth rate, feed efficiency, protein efficiency ratio, protein and lipid retention were analyzed for variance (ANOVA) using SPSS 14.0 for Windows program and followed by Tukey Test at 95% confidence intervals.

3. Results and discussion

The growth pattern and performance of coral trout juvenile fed the experimental diets are presented in figure 1 and table 2. Fish fed diet supplemented with 4% shrimp hydrolyzate had the highest growth response (final weight, WG and SGR) that was significantly different from the other group of treatments (\( P < 0.05 \)). Meanwhile, the growth response of fish fed by the other treatments, including the control treatment, was not significantly different (\( P > 0.05 \)). The survival rate of fish until the end of the experiment was quite high ranging from 90.0 to 95.8% and was not significantly different among treatments (\( P > 0.05 \)). This results showed that the life of the fish was well supported by the experimental conditions. During the experiment, fish mortality occurred due to several fish were lack of appetite in consuming feed, which made them thin and weak.

![Figure 1. Growth patterns of coral trout juvenile fed the experimental diets for 84 days](image)

The specific growth rate (SGR) of coral trout juvenile in this experiment ranged from 1.06 to 1.22%/day, or grew 143.5 - 177.0 % for 84 days of rearing. The daily feed consumption (DFC) reached to 1.7 - 1.8% / day and did not differ significantly among treatments (\( P > 0.05 \)), although in this experiment the fish were fed up to the level of satiation.
Fish hydrolysate supplementation in diet significantly affected feed efficiency (FE) and protein efficiency ratio (PER) of coral trout juvenile (table 2). The increased level of fish hydrolysate supplementation in diet tended to increase FE and PER values. Shrimp hydrolysate supplementation at 4% level resulted in the highest EF and PER (table 2). Meanwhile, although the FE and PER values tended to increase with the increase in tuna hydrolysate supplementation from 2% to 4% in the diet, these values were not statistically significant (P> 0.05). The results of this experiment showed that fish hydrolysate with the optimum level was effective in increasing the feed efficiency of coral trout. This result was also supported by the feed conversion ratio (FCR), where fish fed diet added with 4% shrimp hydrolysate had the best FCR value and was significantly different from the other treatments (P <0.05).

Table 2. Survival, final weight, weight gain (Wg), specific growth rate (SGR), feed conversion ratio (FCR), and protein efficiency ratio (PER) of coral trout juvenile fed the experimental diets for 84 days

| Parameters                 | Control          | Shrimp hydrolysate-2 | Shrimp hydrolysate-4 | Tuna hydrolysate-2 | Tuna hydrolysate-4 |
|----------------------------|------------------|-----------------------|----------------------|--------------------|--------------------|
| Survival (%)               | 90.0 ± 2.5a      | 92.5 ± 6.6a           | 94.2 ± 3.8a          | 95.0 ± 4.3a        | 95.8 ± 2.9a        |
| Final weight (g)           | 18.8 ± 0.9a      | 18.7 ± 0.8a           | 21.3 ± 1.8b          | 18.6 ± 0.4a        | 19.1 ± 1.0a        |
| Wg (%)                     | 147.1 ± 3.9a     | 144.8 ± 5.5a          | 177.0 ± 12.8b        | 143.5 ± 5.8a       | 149.7 ± 10.5a      |
| SGR (%/day)                | 1.08 ± 0.02a     | 1.07 ± 0.03a          | 1.22 ± 0.05b         | 1.06 ± 0.03a       | 1.09 ± 0.05a       |
| DFC (% biomass/day)        | 1.8 ± 0.1a       | 1.8 ± 0.1a            | 1.7 ± 0.1a           | 1.7 ± 0.1a         | 1.8 ± 0.2a         |
| FCR                        | 2.29 ± 0.12c     | 2.36 ± 0.10c          | 1.85 ± 0.08a         | 2.19 ± 0.08hc      | 2.11 ± 0.07b       |
| FE (%)                     | 43.26 ± 2.30ab   | 42.49 ± 1.94a         | 54.22 ± 2.41c        | 45.62 ± 1.60ab     | 47.37 ± 1.52b      |
| PER                        | 1.08 ± 0.04a     | 1.06 ± 0.04a          | 1.33 ± 0.05c         | 1.12 ± 0.03ab      | 1.18 ± 0.03b       |

The proximate composition of fish body, as well as protein and lipid retention are presented in table 3. The dry matter, protein, lipid and ash content of coral trout fed the experimental diets for 84 days was not significantly different (P> 0.05). Protein retention (PR) tend to increase with the addition of fish hydrolysate in the diets. However, only the addition of 4% shrimp hydrolysate produced the highest PR value (24.36%) and was significantly different from the other treatments (table 3). The experimental diets were formulated to have the same protein and lipid content (table 1). Thus, the difference in fish growth and nutrient retention in the fish body were as a response to the addition of fish hydrolysate component in the experimental diets formulation.

Fish hydrolysate which is produced from by-product of fishery processing industries is known to contain high nutritional value. Fish hydrolysate contains hydrolyzed proteins such as peptides and free amino acids that are easily digested and absorbed by fish. Therefore, it can increase fish appetite, growth and immunity [4]. Fish protein hydrolysate was reported to have antioxidant activity and several functional properties that promote fish growth and health [6]. The present study showed that the addition of 4% shrimp hydrolysate to the diet significantly increased the growth (SGR and Wg) of coral trout. The results of the experiment also showed that fish growth tended to increase with the increase in fish hydrolysate supplementation levels, although growth of fish fed by tuna hydrolysate-supplemented diet was not statistically significant at 2% and 4%. Dietary supplementation of 3% fish protein hydrolysate was also reported to be effective in increasing the growth performance of Asian sea bass L. calcarifer both in the nursery and grow-out phases [7]. Furthermore, fish protein
hydrolysate-supplemented diet at level of 5% was also effective in increasing growth performance, feed utilization, and protein digestibility of juvenile red seabream, Pagrus major [8]. There had been found that the addition of 5% and 10% tuna hydrolysate in feed significantly increased the growth of Asian sea bass, L. calcarifer [9]. Dietary low protein content of fish meal supplemented with shrimp hydrolysate at 3% and 4.5% were also reported to be effective in increasing the growth of flounders (Paralichthys olivaceus) which reared for 10 weeks [10].

### Table 3. Proximate composition, protein retention (RP) and lipid retention (RL) of coral trout fed the experimental diets for 84 days

| Parameters       | Treatments                      |          |          |          |          |
|------------------|---------------------------------|----------|----------|----------|----------|
|                  | Control                         | Shrimp hydrolysate-2 | Shrimp hydrolysate-4 | Tuna hydrolysate-2 | Tuna hydrolysate-4 |
| Dry matter (%)   | 26.03 ± 0.35<sup>a</sup>        | 25.97 ± 0.23<sup>a</sup> | 26.09 ± 0.27<sup>a</sup> | 25.98 ± 0.43<sup>a</sup> | 26.13 ± 0.28<sup>a</sup> |
| Protein (% DM)   | 65.09 ± 0.43<sup>a</sup>        | 65.16 ± 0.45<sup>a</sup> | 66.08 ± 0.48<sup>a</sup> | 65.00 ± 0.40<sup>a</sup> | 64.51 ± 0.76<sup>a</sup> |
| Lipid (% DM)     | 7.67 ± 0.36<sup>a</sup>         | 7.63 ± 0.78<sup>a</sup> | 6.77 ± 0.62<sup>a</sup> | 7.49 ± 0.73<sup>a</sup> | 7.14 ± 0.55<sup>a</sup> |
| Ash (% DM)       | 23.28 ± 0.68<sup>a</sup>        | 22.54 ± 0.92<sup>a</sup> | 23.72 ± 0.92<sup>a</sup> | 22.94 ± 0.58<sup>a</sup> | 23.43 ± 0.49<sup>a</sup> |
| PR (%)           | 19.50 ± 0.81<sup>a</sup>        | 19.16 ± 0.95<sup>a</sup> | 24.36 ± 0.53<sup>c</sup> | 20.13 ± 0.89<sup>ab</sup> | 21.05 ± 0.68<sup>b</sup> |
| LR (%)           | 4.76 ± 0.27<sup>a</sup>         | 4.23 ± 1.20<sup>a</sup> | 4.46 ± 1.57<sup>a</sup> | 4.50 ± 1.15<sup>a</sup> | 4.24 ± 1.62<sup>a</sup> |

The data mentioned above shows that the level of fish hydrolysate supplementation which can effectively support growth of fish is likely to vary widely. This could be because of the differences in raw materials and manufacturing processes of fish hydrolysate, which resulted in different chemical characteristics and the active ingredients components of hydrolysate itself [10]. In addition to the manufacturing process, differences in application techniques of fish hydrolysate to the feed could also provide different responses to fish growth. It has been reported that application of fish hydrolysate through a feed coating process was effective in supporting the growth of Asian sea bass [7]. Fish hydrolysate application could also be done by process of feed formulation and was also found to be effective in supporting fish growth performance [8, 11]. The application of fish hydrolysate in feed formulation could also be directed to replace fish meal as a source of protein in feed. Application of fish protein hydrolysate in feed formulation improved the growth performance of orange-spotted grouper, Epinephelus coioides [12]. It was further stated that up to 20% protein from fish meal could be substituted with fish protein hydrolysate without disrupting the growth of orange-spotted grouper. For freshwater fish species, such as tilapia juvenile could utilize fish hydrolysate effectively for its growth and the optimal level of fish hydrolysate in feed was 4.75% [13]. Meanwhile, the addition of 4% salmon hydrolysate to a diet formulated with enzyme treated soybean meal did not provide an increase in growth response of Florida pompano Trachinotus carolinus [14].

Fish hydrolysate with a high content of free amino acids is expected to increase the appetite of fish. However, in the present study, the application of two types of fish hydrolysate (shrimp and tuna hydrolyzate) in diet, each at the level of 2% and 4% did not increase the daily feed consumption (DFC) of coral trout which only ranged from 1.7 - 1.8% of the fish biomass per day (table 2). These values were relatively small when compared to the DFC of hybrid grouper juvenile which reached to 2.7 - 3.5% / day [15]. The low level of feed consumption was indeed related to the relatively slow feeding habits and behavior of coral trout juvenile.

Results of the present study indicated that increasing supplementation levels of fish hydrolysate from 2% to 4% tended to increase feed efficiency (FE) and protein efficiency ratio (PER). However, the best FE and PER were obtained in fish fed diet with 4% shrimp hydrolysate supplementation. Furthermore, supplementation of 3% and 4.5% shrimp hydrolysate in diet with low fish meal content
was reported effective increased the feed utilization efficiency in flaunder (*Paralichthys olivaceus*) [16]. The increase in FE and PER with the addition of fish hydrolysate in feed was also found in juvenile rainbow trout *Oncorhynchus mykiss* [17] and red seabream, *Pagrus major* [8]. Although in general, the addition of fish hydrolysate to feed increases the feed utilization efficiency, however, its effectiveness varies greatly depending on the type of raw materials and the manufacturing process of the fish hydrolysate. In the present study, use of fish hydrolysate for coral trout showed that shrimp hydrolysate supplementation at the level of 4% was more effective in supporting growth of the fish than tuna hydrolysate at the same level. Although, there was also a tendency that increasing the level of fish hydrolysate from 2% to 4% in diet increased feed efficiency and growth of coral trout juvenile.

4. Conclusion

Increasing fish hydrolysate supplementation in diet tended to increase the growth performance and feed efficiency of coral trout. The addition of shrimp hydrolysate in diet at the level of 4% was effective in increasing the growth performance and feed efficiency, and therefore it is recommended for the aquaculture of coral trout.

References

[1] Giri N A, Suwirya K, Marzuqi M 1999 *Jurnal Penelitian Perikanan Indonesia* 5, 38-46
[2] Giri N A, Suwirya K, Marzuqi M 2002 *Indonesian Fisheries Research Journal* 8, 5-9
[3] Marzuqi M, Giri N A, Suwirya K 2007 *Aquacultura Indonesiana* 8, 113-119
[4] Toppe J, Olsen R L, Pefarubia O R, James D G 2018 *A manual on how to turn fish waste into profit and a valuable feed ingredient or fertilizer*. Rome, FAO. p 28
[5] Khosravi S, Bui H T D, Rahimnejad S, Herault M, Fournier V., Kim S S, Jeong J B, Lee K J 2015 *Aquaculture* 435, 371–376
[6] Liaset B, Espe M 2008 *Process Biochemistry* 43, 4-48
[7] Novriadi R, Hermawan T, Ibtisam, Dikurrahman, Kadari M, Herault M, Fournier V, Seguin P 2014 *Jurnal Akuakultur Indonesia* 13, 179–188
[8] Bui H T D, Khosravi S, Fournier V, Herault M, Lee K J 2014 *Aquaculture* 418-419, 11-16
[9] Siddik M A B, Howieson J, Gavin J, Partridge G J, Fotedar R, Gholipourkanani H 2018 *Scientific Reports* 8:15942 | DOI:10.1038/s41598-018-34182-4
[10] Sirichannum M, Tantikitti C, Kortert T M, Krogdahl A, Chotikachinda R 2014 *Aquaculture* 428–429, 195–202
[11] Xu H, Mu Y, Liang M, Zheng K, Wei Y 2017 *Aquaculture Research* 48, 2945-2953
[12] Sheen S S, Chen C T, Ridwanudin A 2014 *J. Aquac. Mar. Biol.* 1, 00006. DOI: 10.15406/jamb.2014.01.00006
[13] Da Silva T C, Rocha J D M, Moreira P, Signor P, Boscolo W R 2017 *Pesquisa Agropecuária Brasileira* 52, 485-492
[14] Novriadi R, Rhodes M, Reis J, Guo J, Swanepoel A, Davis D A 2020 *Journal of Applied Aquaculture*, DOI: 10.1080/10454438.2020.1757010
[15] Giri N A, Muzaki A, Marzuqi M, Sudewi 2020 *IOP Conference Series: Earth and Environmental Science* 584 012030. doi:10.1088/1755-1315/584/1/012030
[16] Gunathilaka B E, Khosravi S, Herault M, Fournier V, Lee C, Jeong J, Lee K 2020 *Aquaculture Nutrition* 26, 1592-1603
[17] Bae J, Azad A K, Won S, Hamidogli A, Seong M, Bai S C 2019 *Fisheries and Aquatic Sciences* 22, 1-8

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