Optimizing protein, lipid and carbohydrate levels in diets for growth of juvenile hybrid grouper \((Epinephelus fuscoguttatus \♀ \times E. lanceolatus \♂)\)

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Abstract. Hybrid grouper \((Epinephelus fuscoguttatus \♀ \times E. lanceolatus \♂)\) that is well known as “cantang” has a good prospect for aquaculture due to its high economic value, fast growing and good acceptance to compound feed. However, information on feed specification for this grouper is still limited. Therefore, this research aimed to find out the good proportions of protein, lipid and carbohydrate in feed for hybrid grouper. Six experimental diets were formulated with protein contents of 48, 44 and 40%, which then combined with lipid contents of 9-17% and carbohydrate contents of 15-32%. Juvenile hybrid grouper of 4.8 ± 0.6 g body weight were stocked into 24 fiber tanks, 400 L in volume, with a density of 40 fish/tank. Fish were fed experimental diet twice every day for 56 days. Results of the experiment showed specific growth rate (SGR) of fish fed 48% protein diet was not significantly different with SGR of fish fed diet containing 44% protein, 14% lipid, 15% carbohydrate and SGR of fish fed diet containing 40% protein, 16% lipid, 15% carbohydrate. The best protein efficiency ratio and protein retention were achieved in fish fed diet containing 44% protein, 14% lipid and 15% carbohydrate as well as in fish fed diet containing 40% protein, 16% lipid and 15% carbohydrate. This result suggested feed containing 44% protein, 14% lipid and 15% carbohydrate or feed containing 40% protein, 16% lipid and 15% carbohydrates was effective in increasing dietary protein efficiency and support good growth for juvenile hybrid grouper cantang.

1. Introduction

Grouper fish is one of the fisheries commodities that have important economic value with high prices and famous as an excellent export products. The successful development of several grouper hatchery technology, including hybrid grouper, has been able to provide a large number of seeds to support the grow-out aquaculture of grouper and increase its production capacity. The hybrid grouper that derived from the crossing between female tiger grouper and male giant grouper \((Epinephelus fuscoguttatus \♀ \times E. lanceolatus \♂)\) in Indonesia is well-known as “cantang” grouper. Cantang hibryd grouper was produced first by Universiti Malaysia Sabah in 2007 [1]. It has been known that hybrid grouper fish are designed to have superiority in growth or in several other characters compared to their parentage [2, 3, 4]. Some of these characteristics are also expected to promote the ability of fish to utilize artificial feeds to be more efficient than that of their parent, this is likely that they have already been more domesticated.
Several studies on the nutritional requirement and feed development of hybrid grouper had been carried out. Feeds containing 50% protein and 14% lipid were reported to be effective in supporting the growth of cantang hybrid grouper [5]. Cantang hybrid grouper are able to utilize squid oil, corn oil, and coconut oil optimally for their growth. Corn oil had been reported to produce the best feed conversion ratio and feed efficiency [6]. Feed formulation for cantang hybrid grouper using vitamin C content of 156 mg/kg of feed had been recommended to obtain optimal growth and normal skeletal development [7]. Because of the high protein content in feed which reached to 50% level, the cost of feed is high and less efficient, thus, an alternative is needed to reduce its costs. The use of soybean meal as a source of protein in feed at 60% level did not affect the palatability of feed, but it significantly decreased the growth rate of cantang hybrid grouper [8], therefore it could not be applied in aquaculture of cantang hybrid grouper.

Thus, further research is required for the development of hybrid grouper feeds which effectively support their growth as well as economically efficient. Feed formulation with an optimal balance between protein, lipid and carbohydrate content is an alternative for achieving an efficient use of dietary protein. The aim of this study was to obtain a feed formula with an optimal proportion of protein, lipid and carbohydrate to promote a good growth of cantang hybrid grouper.

2. Materials and methods

2.1. Hybrid grouper juveniles
A total of 1,500 cantang hybrid grouper juveniles with a weight range of 3-5 g were obtained from the hatchery of the Institute for Mariculture Research and Fisheries Extension (IMRAFE) Gondol, Bali. The fish were kept in a 2 x 2 x 0.75 m fiber tank and fed with a compound feed formulated in the IMRAFE’s Feed Laboratory for the adaptation phase before being used for the experiment.

2.2. Experimental diet
Six experimental diets were formulated with protein content of 40, 44, and 48%, lipid 9 -17% and carbohydrate 15 - 32%. The experimental diets comprised the same total energy content of 4.5 kcal/g of feed (table 1). Feed ingredients were processed into pellets using a pellet machine (Royal, Japan) with a die diameter of 2 mm. The feeds were then dried in an oven at 70 °C. Further, the diets were packed into plastic bags and stored in a refrigerator before and during use for the experiment.

2.3. Feeding experiment
The experiment was done in the IMRAFE’s Wet Laboratory from February to April 2018. Feeding experiment was carried out in 24 fiber tanks, 400 L in volume, equipped with a flow-through water system (6 L/min.) and aeration as oxygen supply. Water from the sea was pumped through a sand filter system before being streamed into the experimental tanks. Cantang hybrid grouper juveniles with an average weight of 4.8 ± 0.6 g were selected and stocked into the experimental tanks with a density of 40 fish/tank. This experiment was designed using a completely randomized design with 6 different feed treatments and 4 replications for each treatment. The fish were fed the experimental diets twice per day, in the morning and evening to the level of satiation. The remaining feed in each tank was collected and dried to determine the amount of feed consumed by the fish. Sampling was done every two weeks by weighing all the fish individually in order to obtain data on growth of the fish. The experiment was performed for 56 days. At the end of the experiment, five fish were sampled from each tank, and then dried with a freeze dryer for the analysis of the chemical composition of the fish's body.

2.4. Observed parameters
The parameters observed included survival rate, final weight (FW), weight gain (Wg), specific growth rate (SGR), daily feed consumption (DFC), feed efficiency (FE), protein efficiency ratio (PER), fish whole body chemical compositions, protein retention (PR) and lipid retention (LR).
Survival Rate (%) = 100 x (Nt/N0)  \hspace{1cm} (1)

Weight Gain (%) = 100 x (Wt – W0) / W0  \hspace{1cm} (2)

Specific Growth Rate (% day-1) = 100 (ln Wt – ln W0) / t  \hspace{1cm} (3)

Feed intake (g)
\[ DFC \ (\%/day) = \frac{\text{Feed intake (g)}}{t \times (W0 + Wt) / 2} \times 100 \]  \hspace{1cm} (4)

FE = Weight gain (g) / feed intake (g)  \hspace{1cm} (5)

PER = Weight gain (g) / protein intake (g)  \hspace{1cm} (6)

Final body protein content (g) – Initial body protein content (g)
\[ PR \ (\%) = \frac{\text{Final body protein content (g)} – \text{Initial body protein content (g)}}{\text{Protein intake (g)}} \times 100 \]  \hspace{1cm} (7)

Final body lipid content (g) – Initial body lipid content (g)
\[ LR \ (\%) = \frac{\text{Final body lipid content (g)} – \text{Initial body lipid content (g)}}{\text{Lipid intake (g)}} \times 100 \]  \hspace{1cm} (8)

Where Nt and N0 are the final and the initial number of fish, respectively. Wt and W0 are the final and initial weight of fish, respectively, and t is the duration of the experiment (days).

Table 1. Composition of the experimental diets (%).

| Ingredients         | Treatments |
|---------------------|------------|
|                     | Diet-1     | Diet-2     | Diet-3     | Diet-4     | Diet-5     | Diet-6     |
| Fish meal           | 52.0       | 52.0       | 48.0       | 48.0       | 44.0       | 44.0       |
| Squid liver meal    | 14.0       | 14.0       | 11.0       | 11.0       | 9.0        | 9.0        |
| Mysid meal          | 10.0       | 10.0       | 9.0        | 9.0        | 8.0        | 8.0        |
| Soybean meal        | 8.0        | 8.0        | 8.0        | 8.0        | 8.0        | 8.0        |
| Dextrin             | 4.7        | 10.7       | 6.5        | 18.1       | 7.2        | 24.4       |
| Vitamin Mix         | 1.3        | 1.3        | 1.3        | 1.3        | 1.3        | 1.3        |
| Mineral mix         | 1.5        | 1.5        | 1.5        | 1.5        | 1.5        | 1.5        |
| Fish oil            | 4.5        | 1.5        | 7.2        | 2.1        | 10.8       | 2.8        |
| Binder/CMC          | 1.0        | 1.0        | 1.0        | 1.0        | 1.0        | 1.0        |
| Filler              | 3.0        | 0.0        | 6.6        | 0.0        | 9.2        | 0.0        |
| Total               | 100        | 100        | 100        | 100        | 100        | 100        |

Proximate compositions of the experimental diets (% DM)

|                | Diet-1 | Diet-2 | Diet-3 | Diet-4 | Diet-5 | Diet-6 |
|----------------|--------|--------|--------|--------|--------|--------|
| C. Protein     | 48.60  | 48.51  | 43.90  | 43.90  | 39.68  | 40.00  |
| C. Lipid       | 12.35  | 9.77   | 14.24  | 8.31   | 16.45  | 9.15   |
| Nitrogen free extract (NFE) | 15.78 | 21.51 | 14.46 | 28.49 | 15.20 | 31.95 |
| C. Fiber       | 3.78   | 3.67   | 4.71   | 3.22   | 4.93   | 4.90   |
| Ash            | 19.49  | 16.54  | 22.69  | 16.08  | 23.74  | 14.00  |
| Gross energy (kcal/g) | 4.62  | 4.62  | 4.47  | 4.53  | 4.48  | 4.54  |

2.5. Proximate composition analysis
Proximate analysis of the experimental diets and the whole body of fish were performed in the Chemistry Laboratory of IMRAFE, Gondol-Bali. The analysis was carried out based on the AOAC International methods [9]. Briefly, water content was determined by drying samples at 105 °C for 16 hours in an oven (Memmert, Germany). Ash content was analysed using a furnace at 550 °C (Carbolite, England). Crude protein content was defined by Kjeldahl method (Kjeltec 8100, Foss). Crude lipid analysis was done by extraction methods using chloroform and methanol. Crude fiber was determined by heating the samples using acids and bases (Fibertec, Foss).

2.6. Data analysis
Data of growth performance and feed efficiency parameters were analyzed using analysis of variance (ANOVA) at 95% confidence intervals. When the effects of the treatments were significant, then the analysis was continued with the Tukey test.

3. Results and discussion
3.1. Growth performance of hybrid grouper
The growth patterns and performance of cantang hybrid grouper fed the experimental diets are presented in figure 1 and table 2. Growth rates of fish fed dietary high carbohydrate (NFE) content (Diet-4 and Diet-6) were seen to decrease on the 28th day of rearing. Growth of fish (Wg and SGR) at the same protein level tend to decrease with the increase in NFE content in feed. The decrease in fish growth was not significantly different (P> 0.05) in fish fed with high protein content (48%) with NFE levels of 15.8% and 21.5%. Meanwhile, increasing NFE content in feed at 28.5% and over, resulted in significantly lower fish growth (P <0.05), both at the 44% and 40% feed protein levels.

Growth of cantang hybrid grouper was relatively fast, and they were very responsive to artificial feeds. In the present study, the specific growth rate (SGR) ranged from 3.08 - 3.51 %/day with a weight gain of 462.7 - 618.0% in all treatments for 56 days of rearing. In a previous study, cantang hybrid grouper with an initial weight of 2.76 g grew 393% for 30 days of rearing with a daily growth rate of 0.36 g/day [6]. The growth rate of the cantang hybrid grouper during the nursery phase reportedly varies between 0.22 – 0.65 g/day for 30 days of rearing with commercial feed [10]. Cantang hybrid grouper with an initial weight of 2.55 g revealed SGR value of 5.76 %/day for 56 days
of experiment when they fed diet with 50% protein content, 14% lipid and 23.6% carbohydrate. In those experiment, it was also reported that dietary lipid at 7% and 14% did not caused in differences in the growth of cantang hybrid grouper [5]. Another study reported that cantang hybrid grouper with an initial weight of 6.34 g showed SGR value of 2.06 %/day or with a daily growth rate of 0.21 g/day for 30 days of rearing [11]. Meanwhile, cantang hybrid grouper with a higher initial weight (23.3 g) grew 148.5% during 14 days of rearing with SGR value of 5.69 %/day [8]. On the contrary, SGR value of 0.80 - 0.83 %/day was obtained in an experiment using a bigger size of cantang hybrid grouper (initial weight of 194 g) for 30 days of rearing [12].

Table 2. Survival, final weight (FW), weight gain (Wg), specific growth rate (SGR), daily feed consumption (DFC), feed efficiency (FE), and protein efficiency ratio (PER) of cantang hybrid grouper fed the experimental diets for 56 days.

| Parameters     | Diet-1           | Diet-2           | Diet-3           | Diet-4           | Diet-5           | Diet-6           |
|----------------|------------------|------------------|------------------|------------------|------------------|------------------|
| Survival (%)   | 98.8 ± 1.4a      | 99.4 ± 1.3b      | 97.5 ± 2.9a      | 98.8 ± 1.4b      | 98.3 ± 2.9a      | 99.4 ± 1.3b      |
| FW (g)         | 34.2 ± 3.3b      | 32.4 ± 1.7b      | 32.4 ± 2.8b      | 27.1 ± 1.4a      | 31.8 ± 3.1ab     | 26.8 ± 1.0a      |
| Wg (%)         | 618.0 ± 69.8b    | 580.6 ± 34.8b    | 580.1 ± 59.3b    | 470.0 ± 29.1a    | 567.0 ± 64.7b    | 462.7 ± 21.8a    |
| SGR (%/day)    | 3.51 ± 0.17b     | 3.42 ± 0.09b     | 3.42 ± 0.16b     | 3.11 ± 0.09a     | 3.38 ± 0.17b     | 3.08 ± 0.07b     |
| DFC (%/day)    | 2.9 ± 0.1ab      | 3.1 ± 0.1bc      | 2.7 ± 0.2a       | 3.2 ± 0.1a       | 2.9 ± 0.1ab      | 3.5 ± 0.1d       |
| FE             | 0.93 ± 0.03ab    | 0.86 ± 0.02b     | 0.98 ± 0.10a     | 0.77 ± 0.03c     | 0.89 ± 0.03ab    | 0.70 ± 0.03d     |
| PER            | 1.92 ± 0.05a     | 1.77 ± 0.03c     | 2.23 ± 0.21b     | 1.74 ± 0.04a     | 2.24 ± 0.06b     | 1.76 ± 0.04a     |

A study on the ratio of NFE/lipid in feeds to the growth of giant grouper (Epinephelus lanceolatus), that is the parent of cantang hybrid grouper had been reported [13]. It was stated that there were no differences in growth of giant grouper at a low NFE/lipid ratio of 0.13 to 1.25. However, the NFE/lipid ratio of 1.88 with 24.43% NFE content and 13.0% lipid significantly inhibited the growth of the giant grouper. Furthermore, the NFE/lipid ratio of 1.3 with 19.23% NFE content and 14.79% lipid was reported to be optimal for the growth of giant grouper. In the present study, the experimental diets had a lipid content of 8.2 - 16.5%. Growth of the fish fed with a combination of 16.5% lipid and 15.2% NFE was better than the combination of 9.2% lipid and 32.0% NFE at the same dietary protein level (40%). These results were in line with the results of an experiment on giant grouper which showed that this fish had limitations in utilizing NFE as an energy source and prefer to use energy from lipid. Similar results were also reported for Wuchang bream fish, Megalobrama amblycephala which grew better when fed with 31% NFE content compared to 45% [14]. However, different results were reported in Asian sea bass, Lates calcarifer, where fish fed with an NFE/lipid ratio varied between 0.47 - 1.21, had no significant differences in SGR values. Those feed contained NFE and fat content of 8.4 - 16.9% and 14-18%, respectively [15].

Data on daily feed consumption (DFC) during the experiment showed that fish fed with higher carbohydrate content showed higher daily feed consumption and significantly different between treatments (P <0.05). These were obtained at both 44% and 40% feed protein levels (table 2). The experimental diets had the same energy content of 4.5 kcal/g. The increase in DFC in treatments with high dietary carbohydrate content indicated that the fish could not utilize energy from carbohydrates optimally, and therefore, they fulfilled their energy from dietary protein or lipid. The same result was also obtained in a previous experiment when cantang hybrid grouper were fed with different protein, lipid and NFE contents. At the same level of dietary lipid, DFC of fish increased when they fed with low protein levels and high NFE [5]. Based on the data of growth performance and feed consumption in the present study, it was found that Diet-3 (44% protein) and Diet-5 (40% protein) were optimal and
resulting in fish growth that was not significantly different from fish fed with 48% protein content (Diet-1 and Diet-2).

3.2. Feed efficiency and protein efficiency ratio

Differences in feed formulations significantly affect on feed efficiency and protein efficiency ratios (P <0.05) (table 2). At the same dietary protein level, fish fed with higher carbohydrate content tended to produce lower FE and the FE values were significantly different both at the 44% and 40% feed protein levels. The values of protein efficiency ratio (PER) showed the same pattern as the values of FE, which decreases with increasing carbohydrate content in feed at the same protein level. Feeds with high carbohydrate content (28.5% in Diet-4 and 32% in Diet-6) produced low PER values (table 2).

The values of FE in cantang hybrid grouper were strongly influenced by protein content and dietary lipid. The increase in protein content or dietary lipid increased the FE values. At the same protein or lipid level, the rise in NFE content resulting in lower FE. Similar results were reported for Wuchang bream, Megalobrama amblycephala [14]. Protein efficiency ratio (PER) was reported low in cantang hybrid grouper [5] and sea bass, Decentrarchus labrax [16] which were fed with high protein content. In the present study, lower PER value was also obtained in fish fed diets containing high protein (48%), but at the dietary protein level of 44% and 40%, the PER values were not different. The PER value was seen to be more influenced by the NFE content, in which feeds with higher NFE at the same protein level resulting in a lower PER value. Low PER values were also found in Wuchang bream, Megalobrama amblycephala which they fed diets with high carbohydrate content [14]. These results implied that the fish tend to utilize energy from protein and could not utilizing energy from carbohydrate maximally to meet their needs. Data on FE and PER in the present study showed that Diet-3 and Diet-5 were efficient and optimal for growth of cantang hybrid grouper.

3.3. Proximate composition of hybrid grouper

The chemical composition of whole body of hybrid grouper, protein retention and lipid retention are presented in table 3. The dry matter content of fish at the end of the experiment was not affected by the experimental diets (P> 0.05). Fish fed with high dietary carbohydrate (28.5% in Diet-4 and 32% in Diet-6) had lower protein content (P <0.05) compared to that of low dietary carbohydrate at the same dietary protein level. Body lipid content of the fish did not showed any clear patterns. However, fish fed with higher lipid content tend to had higher body lipid content as well (Diet-3 and Diet-5). These results were agreed with the results of a previous experiment reported [5], but this phenomenon did not occurred in fish fed with high protein diet (48%). The increase in body lipid content of fish was also found with the rise in the lipid content in diets which were reported in the previous experiment on cantang hybrid grouper [5] as well as giant grouper E. lanceolatus [13].

Table 3. Proximate compositions, protein retention (PR) and lipid retention (LR) of cantang hybrid grouper fed the experimental diets for 56 days.

| Parameters          | Treatments             |
|---------------------|------------------------|
|                     | Diet-1                 | Diet-2 | Diet-3 | Diet-4 | Diet-5 | Diet-6 |
| Dry matter (%)      | 26.2 ± 0.8             | 26.7 ± 0.9 | 27.3 ± 0.7 | 26.7 ± 0.3 | 26.2 ± 0.6 | 26.7 ± 0.1 |
| Protein (% DM)      | 63.0 ± 0.6             | 61.3 ± 0.8 | 62.0 ± 1.3 | 58.9 ± 0.8 | 61.0 ± 0.5 | 58.0 ± 0.6 |
| Lipid (% DM)        | 13.8 ± 0.6             | 14.6 ± 1.2 | 17.0 ± 0.4 | 14.2 ± 0.4 | 19.3 ± 1.1 | 17.7 ± 1.1 |
| Ash (% DM)          | 15.0 ± 0.3             | 14.6 ± 0.8 | 11.8 ± 0.3 | 13.6 ± 0.4 | 11.9 ± 0.9 | 12.9 ± 0.9 |
| Fiber (% DM)        | 1.9 ± 0.3              | 1.7 ± 0.4 | 1.8 ± 0.4 | 2.0 ± 0.2 | 1.1 ± 0.3 | 1.0 ± 0.3 |
| NFE (% DM)          | 6.4 ± 0.4              | 7.8 ± 0.6 | 7.4 ± 0.5 | 11.3 ± 0.8 | 6.7 ± 0.5 | 10.4 ± 0.5 |
| PR (%)              | 32.6 ± 1.3             | 29.6 ± 0.4 | 38.5 ± 3.8 | 30.2 ± 1.1 | 34.6 ± 0.6 | 29.3 ± 0.4 |
| LR (%)              | 37.8 ± 2.8             | 35.1 ± 3.9 | 38.2 ± 4.7 | 35.6 ± 1.1 | 33.3 ± 2.0 | 31.7 ± 2.1 |
Protein retention (PR) and lipid retention (LR) showed the same patterns, which tend to be higher in fish fed with lower carbohydrate content at each level of dietary protein. Lipid retention values were also higher in fish fed with higher lipid content. Data on whole body lipid content of fish and LR values indicated that cantang hybrid grouper tend to store the excess of lipid in their tissues. These patterns were also reported in humpback grouper, *Cromileptes altivelis* [17], tiger grouper, *Epinephelus fuscoguttatus* [18], and red emperor snapper, *Lutjanus sebae* [19].

4. Conclusion
Feed containing 44% protein, 14% lipid and 15% carbohydrate or feed containing 40% protein, 16% lipid and 15% carbohydrate was effective in increasing the efficiency of dietary protein and support good growth for juvenile cantang hybrid grouper.

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