Feed efficiency in the growth of humpback grouper *Cromileptes altivelis* with prebiotics dietary modulation

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Abstract: Energy for fish to carry on with its biological activities and growth comes from food that they eat. In artificial diets, protein become the most expensive source of energy so it is necessary to keep the proportion of protein down to the optimum levels for good growth and feed conversion. In another study, prebiotics resulted in good growth of fish using a manufactured prebiotic supplement. Ideally, we want food material available all year, inexpensive and not be in competition with food material for human consumption. In this study, food formulation with prebiotics made from local products were prepared for fish food. In this study sago was the source of carbohydrates, mackerel and land snails were the source of protein. The formulation was fed to humpback grouper *Cromileptes altivelis* to study the effectiveness on its growth. Ninety humpback groupers (size 9-9.5 cm, weight 10 – 11 g) were reared in 3 fiberglass tanks for four weeks. Each tank had 30 fish, first tank as control without sago and the two tanks with sago in dietary prebiotic pellet. Length and weight gain were recorded every week then growth performance of fish were assessed in the form of Specific Growth Rate (SGR), Food Conversion Ratio (FCR), weight gain daily and length-weight relationship. Food proximate test were carried out separately in the Integrated Chemistry Laboratory, Institut Pertanian Bogor. Data analysis showed a significant difference in the growth performance of humpback grouper through the values of SGR, FCR and average growth daily, between treatment A and B (with sago) and treatment C (control, zero sago). The proximate test displayed lower protein in treatment A (57.95%) and B (55.95%) but higher in treatment C (59.37%), in the contrary to carbohydrate for treatment A (27.80%), treatment B (26%) and treatment C (22.98%).

1. Introduction

The importance of diet for aquacultured fish was investigated for metabolism, replacement of damage body tissues and growth. The quality of food depends on the composition of nutrients, such as protein, carbohydrate and fat for the growth of aquacultured fish [1].

The concept of prebiotic was introduced initially to human colonic microbiota to improve host health [2]. An application of probiotic, prebiotic and symbiotic was reviewed [3] and studied [4,5,6] showed positive growth development of aquatic organisms. Prebiotics stimulate the growth of gut commensal bacteria of fish, thus improving health. In this study mackerel, land snail and sago, all local ingredients were used for dietary modulation. The use of inexpensive materials to result in more
effective aquaculture is the goal. Although many research studies on prebiotics substances applied in aquaculture organisms [7], no study of prebiotics in humpback grouper has yet been published. In this study, the efficiency of prebiotic modulation given to humpback grouper (Cromileptes altivelis) was studied to see the effect of its growth performance.

2. Materials and methods

2.1. Preparation of diet

Dietary prebiotic preparation using mackerel (Decapterus sp.) flour, land snail (Achatina sp.) flour, sago (Metroxylon sp.) flour, tapioca (Manihot utilissima) flour, mix vitamins Bio Fish® and Fish oil Omega 3 Plus DHA®. The amount of all the ingredient were determined after a preliminary formula test was conducted to get a right composition, durability in the water and the size of pellets. Prepared diets compositions were using Person Square method [8] as shown here.

| Food Materials  | Treatment A | Treatment B | Treatment C |
|-----------------|-------------|-------------|-------------|
| Mackerel flour  | 1160 g      | 1160 g      | 1160 g      |
| Land snail flour| 580 g       | 580 g       | 580 g       |
| Sago flour      | 195 g       | 130 g       | 0 g         |
| Tapioca Flour   | 65 g        | 130 g       | 260 g       |
| Fish Oil        | 5 g         | 5 g         | 5 g         |
| Mixed Vitamins  | 10 g        | 10 g        | 10 g        |
| Water           | 1500 ml     | 1500 ml     | 1500 ml     |

2.2. Preparation of rearing tanks, experimental fish and diet

Fish were held in 3 fiber glass rectangular tanks which were 3.0 x 1.5 x 0.75 m. The tanks were cleaned with chlorine to remove bacteria on its walls. Fish were acclimatized for one day and were not fed. The water quality parameters in the tanks were maintained in a similar range in that found in nature and measured every week to be compared to the ideal condition.

A total of 90 fish were used length 9.0–9.5 cm and weight 10–11 g randomly distributed into three experimental groups, each tank having 30 individuals. Fish were fed ad libitum, three times a day with 10% of average total weight per day. The remaining food and feaces were taken every morning before feeding time. Food weight for each treatment during the experiment is shown in the table below.

| Treatment | Treatment A | Treatment B | Treatment C |
|-----------|-------------|-------------|-------------|
| Week 1    | 240.8 g     | 229.6 g     | 221.2 g     |
| Week 2    | 325.5 g     | 313.6 g     | 272.3 g     |
| Week 3    | 385.7 g     | 366.8 g     | 294.7 g     |
| Week 4    | 433.3 g     | 396.2 g     | 320.6 g     |
A proximate test of food was done for protein, fat, carbohydrate, water content, ash and (crude) fibre.

2.3. Growth performance
To analyze growth performance, Relative Growth (RG), Specific Growth Rate (SGR), Food Conversion Ratio (FCR) were determined using the formulae described [9], [10]. To calculate SGR using equation as follow; \( SGR = \left( \frac{\ln W_t - \ln W_0}{t} \right) \times 100\% \); where \( W_t \) = Final weight, \( W_0 \) = initial weight, \( t \) = time of experiment. Food Conversion Ratio (FCR) is the amount of feed consumed by fish (g) to increase 1 g of fish weight, calculated to measure fish growth rate and consumed feed. The equation used [11]; \( FCR = \frac{F}{W_t + W_m + W_o} \); where, \( F \) is the amount of feed given during treatment (g), \( W_0 \) is initial fish biomass (g), \( W_m \) is biomass of dead fish during treatment (g) and \( W_t \) is final biomass of the fish (g).

2.4. Data analysis
The results were analyzed using SPSS. A one-way ANOVA was used to compare the data on growth performance, Relative Growth, Length – Weight Relationship and SGR. Multiple comparisons were analysed with Tukey HDS and Duncan Test. FCR was analyzed using Excel.

3. Result and discussions
No fish were found dead during the period of the experiment. The water quality in the tank was maintained properly during the experiment and measurement parameters can be seen in Table 3 below.

| Parameters     | Lab measurement | Standard optimal | References |
|----------------|-----------------|------------------|------------|
| Temperature (°C) | 28.1 – 29.9     | 27 - 30          | [12]       |
| pH             | 8.1 – 8.4       | 7 – 8.5          | [13]       |
| DO (mg/l)      | 4.7 – 5.8       | 4.0 – 6.0        | [14]       |
| Salinity (ppt) | 33.5 – 34.0     | 30 - 35          | [15]       |

Specific Growth Rate (SGR) showed a similar tendency to percentage of weight which increased in all three treatments, Treatment A was the highest (9.33%) followed by treatment B (7.45%) and treatment C (3.82%), a significant difference between treatments.

Weight Increase differed significantly p<0.05 in the growth performance measurement. The growth of humpback grouper was higher in weight than in length. The percentage of Specific Growth Rate had a similar result above showing the growth performance were significantly higher in the feeding treatment with sago. A worked with juvenile humpback grouper and found that dietary food and high water quality maintenance functioned as an energy supply for growing and life maintenance [16].

Length and weight relationship used as factors to show the changing of dimensions such as weight of an individual as well as relative growth pattern of the population. The graphic below shows the lines of treatment A and B have a similarity compared with treatment C.
Weight-length relationship of humpback grouper shows changes of dimensions as well as condition factors on different treatments between feeding with and without sago. This was shown by the relative growth of those group of fish in this study. A study on sardines [17], that conversion of length to weight or otherwise was related to a condition factor or the fatness of the organism. Humpback grouper shows the group of fish treated with prebiotics diets were fatter than the group without sago.

The result of FCR during the experiment for each treatment as shown in the figure below. The values of FCR in the first week was low then increased to the second week for all treatments. It then decreased in week 2 until 4 for treatment C. Treatment A and B experienced an increase until week 3 then fell as well in week 4. FCR in average to treatment A, B and C was 3.44, 3.73 and 5.70 respectively.
This study found the values of FCR on average between treatment A and B (with sago) and treatment C (control, zero sago) was significantly different in the growth performance of humpback grouper. However, no significant different was detected between treatment A and B where both had sago with different amounts in their diet. This means feeding prebiotics results in high efficiency of feed utilization. Feed is the most significant variable cost in aquaculture and waste must be minimized. Usually more than 50% of production expenses in intensive fish farming are related to feed. Thus, the inclusion of dietary prebiotics led to more benefit in the food.

The composition of protein and carbohydrate in the proximate test was contrary to each other. Protein in the treatment A and B with the inclusion of dietary prebiotics were lower while in the treatment C without it was higher but not in the composition of carbohydrate as in the Table 4 below.

| Parameters        | Treatment A (%) | Treatment B (%) | Treatment C (%) | Method                        |
|-------------------|-----------------|-----------------|-----------------|-------------------------------|
| Water Content     | 5.26            | 8.88            | 9.80            | SNI 01.2891-1992              |
| Ash Content       | 5.04            | 4.92            | 4.48            | SNI 01.2891-1992              |
| Fat Content       | 4.01            | 4.03            | 3.17            | AOAC (2005) Method 2003.0     |
| Protein Content   | 57.95           | 55.95           | 59.37           | IK NO. LT-III-5.4.1.3-G       |
| Carbohydrate      | 27.80           | 26.00           | 22.98           | IK NO. LT-III-5.4.1.3-F       |
| Crude Fiber       | 5.56            | 4.10            | 3.80            | SNI 01.2891-1992              |

The fish fed with modulate diet containing prebiotic supplement gave an indication of better growth performance compare to those in the control group. Fish grow to its optimum at 42.55% of protein in their diet [18], however in this study where the protein was 59.37% growth of fish fed with zero sago was significantly different to those fish fed with sago. The proximate test displayed lower protein in treatment A (57.95%) and B (55.95%) compared to group of fish in treatment C that was higher (59.37%). Carbohydrate content, in the contrary has higher in treatment A (27.80%), compare to treatment B (26%) and treatment C (22.98%). This also stresses that growth of fish cannot rely on protein intake alone, but also fitness and health condition of the fish.
Sago has polyphenols like tannins and flavonoids which function as antioxidants that neutralize potentially harmful molecules known as free radicals. As high levels of free radicals in body can cause cellular damage. In a study on rats fed with sago-rich diets [19] the results showed fewer signs of free radical damage at higher antioxidant levels. It also reduced risk of disease associated with narrowed arteries due to cholesterol build up called atherosclerosis, compared to mice fed with low-sago diets. A research study about polyphenols showed the abundance of polyphenols in diets can cause immunity improvement and reduce inflammation [20].

Prebiotics were known as non-digestible food ingredients that stimulate the growth and health of living organisms. Also, prebiotic supplements directly modulate the endogenous flora in the gastrointestinal tract by producing or influencing enzyme activity [21]. Digestive tract functions to breakdown food stuffs into molecules compatible with absorption across the epithelial border of the gastrointestinal tract [22] with the aid of digestive enzymes in the intestine of fish. Sago has approximately 7.5% resistant starch that passes through digestive tract undigested [23] and feeds healthy gut bacteria that break down resistant starch and produce short chain fatty acids (SCFA) [24].

4. Conclusions
The result of this present study strongly supports the efficiency of supplemented diets with prebiotics. A positive effect was shown in the values of Specific Growth Rate, percentage of Weight Increased, relationship of weight and length, and FCR. However, further study needs to be established to observe the performance of longer rearing periods for the various benefits in disease resistance and the amount of sago in the composition of diet on the survival rate.

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