Sea lettuce (*Ulva lactuca*) meal combined with soybean meal in a low fishmeal diet enhanced the growth and feed intake of cultured golden rabbitfish (*Siganus guttatus*) during the nursery stage

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**Abstract.** A preliminary feeding trial was conducted to evaluate the effect of utilizing a high level of sea lettuce (SL), *Ulva lactuca* meal, as a dietary ingredient on the growth and biochemical composition of the golden rabbitfish, *Siganus guttatus*. Two treatments included one diet containing high SL meal combined with soybean (PP) and another diet that used fish meal as the primary protein source (FM). Golden rabbitfish were stocked into 8 of 120 L fiberglass tanks with a density of 20 fish/tank and fed for 15 weeks. The weight gain of the fish-fed PP diet was 453% higher than those provided with FM (343%). Similarly, the SGR of fish fed the PP diet was higher (1.40 %/d) compared to the PF diet (1.22 %/d). The FCR of the FM diet was lower (1.3) compared to the PP diet (1.6). Fish fed the FM diet contained a higher ash content than those fed the PP diet (14.9 vs 10.1%). Crude protein content in body fish was relatively similar for both diets. Including sea lettuce meal at 30% combined with a high level of soybean meal in a low fishmeal diet, they produced better growth performances of rabbitfish during the nursery stage.

**1 Introduction**

Feed represents up to 60% of the cost of aquaculture production [1], and the continuously increased price of some vital aquafeed ingredients, such as fish meal, soybean meal, and fish

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Oil has driven the need for alternative sources to decrease feed costs and make aquaculture more sustainable [2, 3]. For the last two decades, studies have focused on evaluating alternative ingredients for aquafeed, including terrestrial and marine origins such as seaweed. Seaweed has been used as fresh feed for aquatic herbivorous species like golden rabbitfish *Siganus guttatus* [4] and abalone *Haliotis squamata* [5]. Seaweed has also been utilized as a feed ingredient with various functions, including as a carbohydrate source [6], functional feed [7], and as a binder [8]. Generally, seaweed contains many essential minerals [9] and amino acids except tryptophan [10].

Sea lettuce, *Ulva lactuca* (local name called *selada laut*), is an edible green macroalgae among the dominant microalgae grown along the Indonesian coastal region. Seaweed Ulva grows faster and can reach a rate of up to 5.22% day⁻¹ [11] and quickly grows in large biomass [12] using inexpensive fertilizers [13]. Biomass of Ulva majority contained carbohydrates, including cellulose, a structural polysaccharide, starch as the storage polysaccharide, and ulvan as the matrix polysaccharide [14]. *Ulva* has crude protein 7.13 – 27.0%, lipid 0 – 2%, carbohydrate 50 – 61.5%, and ash 11.0 – 49.6% [15, 16, 17, 18, 19]. Besides its nutritional aspects, Ulva is also widely used for bioremediation because of its high growth rate and ability to absorb nutrients in broad environmental conditions [20].

Several studies on the use of Ulva have been reported on aquatic species, including freshwater species of grey mullet (*Mugil cephalus*) fingerlings [21], Nile tilapia, *Oreochromis niloticus* [22], rainbow trout, *Oncorhynchus mykiss* [23] and combination of sea lettuce and red algae *Pterocladia capillacea* for juvenile of gilthead seabream (*Sparus aurata*) [24]. In addition, [25] reported that including *Ulva rigida* in the diet of European seabass juveniles has no adverse effects on fish performance.

To evaluate sea lettuce meal as an alternative feed ingredient, a feeding trial was accommodated to assess the effect of a high inclusion level of sea lettuce meal combined with soybean meal in a low fish meal diet on growth, feed utilization, and biochemical composition of cultured golden rabbitfish, *Siganus guttatus*.

### 2 Materials and methods

#### 2.1 Sea lettuce meal and experimental diets

Dried sea lettuce used in the experiment was obtained from Nusa Lembongan Island, Bali. Dried sea lettuce was dried again in the Laboratory of Fish Nutrition and Feed Technology of RICAFE using a conventional oven and then grounded using a hammer mill.

The feeding trial was designed into a Completely Randomized Design, two treatments with four replicates. The two experimental diets were formulated using different protein sources to contain the same protein level. One diet used a high inclusion level of fish meal (32%) as the primary protein source (FP), and the other diet was formulated with low fishmeal at 5% and a high level of sea lettuce meal at the rate of 30% combined with soybean meal at 42% as the major plant ingredients (PP). Wheat gluten was supplemented 6% in the two diets to meet the desired levels of the tested diet to be approximately 33% [26] and lipid content around 9-10% [27]. Fish oil was used as a lipid source for the FP diet, while the PP diet was added to minimize animal-origin ingredients contained in the PP diet. As the binder, both diets were incorporated with Gracilaria at a level of 6.4%. Tables 1 & 2 present the analyzed proximate composition of the two experimental diets.

Experimental diets were made by first weighing all ingredients used and mixing all dried ingredients thoroughly until homogenous, then adding oil sources and mixing again. Water was added to moist the dough, approximately 30-35% of the dry ingredients [28], then pelletized using a pellet machine (Hiraga, Co. Ltd, Kobe, Japan). The pellet produced was
Table 1. Formulation of experimental diets (%)

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Experimental diets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FP¹</td>
</tr>
<tr>
<td>Fishmeal</td>
<td>32</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>0</td>
</tr>
<tr>
<td>Wheat gluten meal</td>
<td>6</td>
</tr>
<tr>
<td>Rice bran meal</td>
<td>44</td>
</tr>
<tr>
<td>Sea lettuce meal</td>
<td>0</td>
</tr>
<tr>
<td>Sargassum meal</td>
<td>6</td>
</tr>
<tr>
<td>Gracillaria meal</td>
<td>6.4</td>
</tr>
<tr>
<td>Palm oil</td>
<td>0</td>
</tr>
<tr>
<td>Fish oil</td>
<td>4</td>
</tr>
<tr>
<td>Stress off</td>
<td>0.1</td>
</tr>
<tr>
<td>Mineral mix</td>
<td>0.5</td>
</tr>
<tr>
<td>Vitamin mix</td>
<td>1</td>
</tr>
</tbody>
</table>

¹Diet containing high sea lettuce meal combined with soybean
²Diet used fish meal as the major protein source

Immediately steamed for three minutes and then dried. Based on the fish size, the diet used for pelletizing was 1.1 and 2.1 mm. Diets were dried until the moisture content was less than 12%. The two diets were stored in a cool room at around 18°C during the feeding trial.

Table 2. Proximates content of the experimental diets

<table>
<thead>
<tr>
<th>Nutrient (%)</th>
<th>Experimental diets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FP¹</td>
</tr>
<tr>
<td>Moisture</td>
<td>5.6</td>
</tr>
<tr>
<td>Crude protein</td>
<td>34.1</td>
</tr>
<tr>
<td>Lipid</td>
<td>10.1</td>
</tr>
<tr>
<td>Ash</td>
<td>14.6</td>
</tr>
<tr>
<td>Fiber</td>
<td>14.7</td>
</tr>
<tr>
<td>NFE</td>
<td>18.9</td>
</tr>
</tbody>
</table>

¹Diet containing high sea lettuce meal combined with soybean
²Diet used fish meal as the major protein source

2.2 Fish and feeding trial

The third generation of golden rabbitfish was produced at the Installation of the rabbitfish hatchery of RICAFE located in Barru District, South Sulawesi. The fish's mean length and body weight at the initial feeding trial were 5.4±0.1 g and 6.3±0.1 cm, respectively. Fish were stocked into 8 of 120 L fiberglass tanks with a density of 20 fish/tank. Diets were fed to satiation four times daily at 0700, 1000, 1300, and 1600 for 15 weeks. The uneaten diet was collected, dried, and weighed to calculate feed intake. The water supply of the tank flowed through an aeration system. All tanks were cleaned up daily from fesses and other contaminants by siphon.

Growth was monitored by weighing and measuring the length of the fish every two weeks. Variables observed were growth, feed intake, feed conversion ratio (FCR), and biochemical composition of fish at the end of the feeding trial.
2.3 Samples and biochemical analysis

Ten fish were collected for whole body and muscle samples at the end of the feeding trial. Muscle samples were prepared by cutting the entire fillet. Proximate analysis of diets, whole-body fish, and muscle were performed according to [29]. Analysis of crude protein was performed following the micro-Kjeldahl procedure, and lipid analysis was carried out by extracting the samples with petroleum benzene using the Soxtherm apparatus. Dry matter (DM) by oven-drying at 105 °C for 16 h using oven (Memmert, Germany). Ash was analyzed using a muffle furnace at a temperature of 550°C (Barnstead, Thermolyne, CA, USA).

2.4 Calculation and statistical analysis

The parameters observed were survival rate (SR), weight gain (WG), specific growth rate (SGR), feed utilization, including feed intake (FI), and feed conversion ratio (FCR). Each of the parameters observed used the below equation for calculation:

$$WG \ (g \ fish^{-1}) = 100 \times (final \ weight \ - \ initial \ weight) / (initial \ weight) \quad (1)$$

$$SGR \ (% \ day^{-1}) = 100 \times (ln \ W_f \ - \ ln \ W_i) / T \quad (2)$$

$$SR \ (%) = 100 \times (final \ number \ of \ fish \ / \ initial \ number \ of \ fish) \quad (3)$$

$$FCR = feed \ intake \ (g \ dry \ weight) / total \ body \ weight \ gain \ (g \ wet \ weight) \quad (4)$$

Where $W_i$ is the average initial weight of the fish, $W_f$ is the average weight at the end of the trial, and $T$ is the number of days between weight measurements [30].

Data on growth performances, feed utilization, and proximate composition of whole body fish and fillet were statistically analyzed using a $t$-test (n=4) using SPSS 25 version (SPSS, Inc., Chicago, Illinois, USA). The level of significance was defined as 0.05.

3 Results and discussion

After 15 weeks of the feeding experiment, fish fed the two tested diets grew linearly, as demonstrated by Figure 1. The final weight of the fish-fed PP diet was 30.4±1.5 g, significantly higher than the group-fed FM diet (23.9±4.6 g). The WG of fish fed the PP diet was 453%, significantly higher than that of fish fed the FM diet, which was 343%. Similarly, fish that consumed the PP diet had higher SGR (1.40 %/d) and significantly differed from fish fed the FM diet (1.22%/d), as illustrated in Table 3. The higher growth rate of fish consumed the PP diet, which contained major plant ingredients and low fishmeal, indicated that golden rabbitfish can utilize the PP diet better than when the diet contained a higher level of 32% fishmeal as a protein source.

In nature, rabbitfish consume aquatic plants, particularly macroalgae or seaweeds, up to 65.9%, 9.8% animal origin, including mollusk, and the rest is semi-digested feed [31]. In this study, the PP diet contained a high portion of 42% soybean meal and a low level of 5% fishmeal as protein sources combined with 30% sea lettuce meal as the primary carbohydrate source produced better growth responses compared to the FM diet containing a high level of fishmeal. This indicated that the major plant-based diet was more suitable for the golden rabbitfish to support their growth than the fishmeal-based diet, mainly when the ingredient was a combination of soybean and sea lettuce meal. Soybean meal as a protein source has been widely investigated for various aquatic species [32; 33] and become one of the primary protein sources used in commercial aquafeed in Indonesia after fishmeal [34]. Our recent
study demonstrated that sea lettuce as fresh feed, when used as a sole feed, did not promote golden rabbitfish to grow normally but stimulated a bright color. However, combining fresh sea lettuce and an artificial diet at 50-75% gave a better growth performance than the golden rabbitfish fed only an artificial diet [4].

**Fig. 1.** Pattern of growth of juvenile golden rabbitfish after a 15-week feeding trial. FM = Diet used fish meal as the primary protein source; PP = Diet containing high sea lettuce meal combined with soybean. Value in each line with different alphabets indicated significant differences (p<0.05)

**Table 3.** Survival rate, weight gain, and specific growth rate of golden rabbitfish fed two tested diets.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Tested diets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FM</td>
</tr>
<tr>
<td>Initial weight (g)</td>
<td>5.4±0.1a</td>
</tr>
<tr>
<td>Final weight (g)</td>
<td>23.9±4.6a</td>
</tr>
<tr>
<td>Initial weight (cm)</td>
<td>6.3±0.1a</td>
</tr>
<tr>
<td>Final weight (cm)</td>
<td>10.3±0.5a</td>
</tr>
<tr>
<td>Survival rate (%)</td>
<td>92.5±2.9a</td>
</tr>
<tr>
<td>Weight gain (%)</td>
<td>343.7±66.8a</td>
</tr>
<tr>
<td>Specific growth rate (%/d)</td>
<td>1.22±0.15a</td>
</tr>
</tbody>
</table>

All values are expressed as mean ±SD, n=4. FM = Diet used fish meal as the major protein source; PP = Diet containing high sea lettuce meal combined with soybean. Diets within rows annotated with the different superscript letters are significantly different (p<0.05)

The SRs were 100% during the feeding trial in fish fed the PP diet, or no mortality was recorded. In contrast, mortalities of fish given the FM diet were recorded since the first three and continued until the 15-was illustrated in Figure 2. The number of dead fish found in four tanks of FM treatment was six fishes, contributing to 92.5% SR on average. However, this SR was not significantly different between the two groups (Table 3). The cause of the mortality was suspected of the fish not consuming the diets properly and further losing weight. No clinical symptom caused by disease infection was observed during the feeding trial.
Fig. 2. Pattern of survival rate (%) after 15-w feeding trial. FM = Diet used fish meal as the primary protein source; PP = Diet containing high sea lettuce meal combined with soybean. Value in each line with the same alphabetic indicated no significant differences (p<0.05)

Besides macronutrients in sea lettuce [35; 4], it also contains functional foods with essential health functions. Like other seaweeds, Ulva also contains a typical polysaccharide called Ulvan, a water-soluble sulfate polysaccharide. The ulvan has several vital functions, such as antioxidants [36; 37], pathogenic antimicrobials [38], and anticancer [39]. If the ulvan is degraded, it will produce a simpler polymer called rhamnose and sulfated oligosaccharides [40]. The higher growth rate of fish fed the PP diet indicated that the fish of the group were in good health partly due to the positive effect of the presence of sea lettuce meal in the PP diet. These better growth and survival performances of fish fed the PP diet also revealed that golden rabbitfish, as herbivorous species, utilized plant ingredients better than a fishmeal-based diet.

Table 4 presents feed intake, and FCR, where fish consumed the PP diet had significantly higher feed intake than fish given the FM diet. In contrast, fish fed the FM diet had a better FCR of 1.3 than the PP diet (1.6). A combination of sea lettuce meal with soybean seemed preferable to golden rabbitfish compared to rice bran, even when formulated with a high amount of fishmeal.

The proximate composition of the whole body and muscle of golden rabbitfish after fed the two diets are demonstrated in Table 5. Crude protein, fiber, and NFE content in the entire body and muscle were not significantly different (P>0.05). However, lipid content in the entire body of fish in the FM diet was significantly lower (P<0.05) than fish fed the PP diet, whereas the ash content of fish in the FM diet was significantly higher than (P<0.05) fish fed the PP diet. The differences in lipid and ash content found in the whole body were not observed in the muscles of fish fed the two diets.

Table 4. Feed intake and FCR of golden rabbitfish during 15 weeks of nursery

<table>
<thead>
<tr>
<th>Variable</th>
<th>Tested feed</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FM</td>
<td>PP</td>
</tr>
<tr>
<td>Feed intake (g/fish)</td>
<td>30.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>49.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>FCR (g/g)</td>
<td>1.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

All values are expressed as mean ±SD, n=4. FM = Diet used fish meal as the major protein source; PP = Diet containing high sea lettuce meal combined with soybean. Diets within rows annotated with the different superscript letters are significantly different (p<0.05)
The higher ash content in the body of the fish-fed FM group was in line with the higher ash content in the FM diet. The ash content (14.9%) in the FM diet was high, coming from the fish meal as the primary ingredient with rice bran, compared to the PP diet, which contained most soybean and ulva meal. Our previous study found a similar trend using a diet formulated with 35% fishmeal containing 14.0% ash content produced fish with 14.7% ash content [41]. Furthermore, higher lipid content in fish fed the P, P diet, which was 18.1%, was suspected partly due to the higher carbohydrate content (38.8%) due to higher plant ingredients incorporated into the diet. However, the fillet lipid concentration did not differ between the two fish groups.

Utilization of high sea lettuce combined with high soybean meal, which produced higher growth performances observed in this study, revealed that sea lettuce is promising to be included in the diet for juveniles of golden rabbitfish.

4 Conclusion

A low fishmeal diet containing a majority of soybean meal and sea lettuce meal (PP diet) produced better performances in golden rabbitfish in terms of SGR (1.44%/d) and feed intake (49.8 g) than a fishmeal-based diet. Golden rabbitfish fed the PP diet had lower ash and lipid content in the whole body. Sea lettuce, as an emerging ingredient for aquafeed can be incorporated at a high level of 30% in the diet of golden rabbitfish, *S. guttatus*, during the nursery stage.

This study was financially granted by the Australian Center for International Agricultural Research (ACIAR) through a collaborative project entitled Accelerating the Development of Finfish Mariculture in Cambodia through South-south Research Cooperation with Indonesia (FIS/20016/124). The authors thank the technicians (Hariano and Waldy Zaenal) and analysts (Rosni and Dian Wahyuni) of RICAFE, MMAF, for their assistance.

References