Effects of *Ulva rigida* and *Cystoseira barbata* Meals as a Feed Additive on Growth Performance, Feed Utilization, and Body Composition of Nile Tilapia, *Oreochromis niloticus*

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**Abstract:** In a 12-week feeding trial, the effect of 2 algae meals (*Ulva rigida* or *Cystoseira barbata*) on feed intake, growth, and nutrient utilization of juvenile Nile tilapia, *Oreochromis niloticus*, was investigated. This was the first attempt to utilize *Cystoseira barbata* as a feed supplement for Nile tilapia. The fish were fed to apparent satiation with formulated diets supplemented with various levels of *Ulva* meal (5%, 10%, or 15%) or *Cystoseira* meal (5%, 10%, or 15%). A diet without algae meal served as a control diet. The highest values for weight gain were for fish fed the 5% *Cystoseira* diet, control diet, and 5% *Ulva* diet (156%, 151%, and 150%, respectively), but the values were not significantly different (P > 0.05) compared to other treatments, except for the fish fed on the 15% *Ulva* diet (P < 0.05), which exhibited the lowest weight gain. Fish fed the diet containing 15% *Ulva* meal showed the poorest feed conversion ratio (FCR). Protein and energy utilization tended to decrease in the groups fed the algae meals at the highest supplementation level of 15%. Carcass lipid levels decreased with increasing levels of *Ulva* meal, while an increase in carcass lipid level with increasing levels of *Cystoseira* meal was observed (P < 0.05). The results suggested that *Ulva rigida* or *Cystoseira barbata* meals could be used in small percentages in tilapia diets.

**Key Words:** *Oreochromis niloticus*, algae meal, *Ulva rigida*, *Cystoseira barbata*, feed supplement, growth performance, feed utilization, body composition
of every possible natural resource as a potential ingredient in aquafeed preparation. Algae have received attention as suitable alternative protein sources for farmed fish since their protein content and production rate are high. Macro- and microalgae, such as Ulva, Ascophyllum, Laminaria, Undaria, Porphyra, Spirulina, and Chlorella, have been evaluated as feed additives in earlier studies. The addition of small amounts of algae meal to fish diets resulted in considerable effects on growth (2-4), feed utilization (4), lipid metabolism (2,4-8), body composition (2,7,8), stress responses (4), liver function, disease resistance (9,10), and carcass quality (3,8). Ulva sp. is one of the most commonly used macroalgae in aquaculture since it contains protein, pigments, vitamins, minerals, and unknown growth factors (2,3,11). Cystoseira barbata have never been used as a feed component in aquafeeds. Ulva rigida and Cystoseira barbata are widespread along the coast of the Aegean Sea and the Dardanelles in Turkey. Tilapia is emerging as a new fish species for large-scale commercial production in Turkey. The objective of this study was to examine and evaluate the growth performance, feed utilization, and body composition of Oreochromis niloticus when fed diets supplemented with various levels of macroalgae meals, namely Ulva rigida and Cystoseira barbata, as feed additives so as to determine the optimal level of dietary algae meal supplementation.

Materials and Methods

Experimental fish, rearing condition, and feeding regime

Juvenile Oreochromis niloticus were obtained from the Faculty of Fisheries, Çukurova University, Turkey, and acclimated to laboratory conditions for 2 weeks while fed a commercial diet (crude protein: 40%, crude lipid: 10%). Three replicate groups of 10 fish were randomly stocked in 60-l glass aquariums. The fish were exposed to a normal photoperiod for 12 weeks (9 h light, 15 h dark). Water temperature, dissolved oxygen, and pH were maintained at 26.0 ± 1.0 °C, 7.6 ± 1.0 mg/l, and 8.0 ± 0.2, respectively. Fish were fed to apparent satiation 3 times a day (0830, 1230, and 1630). On a daily basis, feces and pellet residues were removed by siphoning and 10% of the water in each aquarium was exchanged. Fish were weighed and recorded every 2 weeks from the beginning of the study. Before weighing, fish were starved for 24 h, allowing the gut to empty.

Experimental Diets

The pre-weighed dry ingredients were carefully mixed using a laboratory food mixer with the separate addition of the oil and vitamin/mineral premix. The mixtures were primed with water to yield a suitable mash for extrusion. The diets were extruded through a series of 2-mm die holes and then dried at 40 °C in a fan-assisted drying cabinet.

An algae-free control diet (C) and 6 experimental diets, including varying levels of Ulva meal (5% (U5); 10% (U10), and 15% (U15)) or Cystoseira meal (5% (C5); 10% (C10), and 15% (C15)) were formulated. Formulation and chemical composition of the experimental diets are shown in Table 1a and 1b, respectively.

Green macroalgae (Ulva rigida) and brown macroalgae (Cystoseira barbata) were freshly obtained together with other sea weeds and various algae species from the near-shore waters of the Dardanelles. Ulva rigida and Cystoseira barbata samples were thoroughly washed with sea water, dried in a drying cabinet at 40 °C for 48-72 h, and fine-milled with a laboratory blender. Finally, the chemical compositions of the samples were determined and are given in Table 2.

Chemical Analyses

All ingredients, experimental diets, and fish samples were analyzed for chemical composition according to AOAC (12). Moisture, ash, crude protein, and total lipids were determined by oven drying at 105 °C, the ignition of samples in a muffle furnace at 550 °C overnight for 12 h, using the Kjeldahl method after acid digestion, and the Soxhlet method, respectively. Crude fiber was determined by acid alkali hydrolysis and ignition of the dried sample for 3 h. Nitrogen-free extract (NFE) was calculated by subtracting the sum of ash, crude protein, lipid, and crude fiber from 100.

Data processing and statistical analysis

Fish performance, in terms of weight gain (WG), feed intake (FI), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), and nutrient retention efficiency, was determined using the following formulae:
WG (%) = \[(\text{final weight (g)} - \text{initial weight (g)}) / \text{initial weight (g)}\] × 100

FCR = feed intake (g) / weight gain (g)

FI (%) = (daily feed intake (g) × 100) / biomass (g)

SGR (% day⁻¹) = 100 × [(ln final fish weight) - (ln initial fish weight)] / experimental days.

Apparent net protein utilization (ANPU) (%) = [(final body protein (g) - initial body protein (g)) / dietary protein consumption (g)] × 100

Apparent net energy utilization (ANEU) (%) = [(final body energy (MJ) - initial body energy (MJ)) / dietary energy consumption (MJ)] × 100

Protein utilized kg⁻¹ growth (g) = (dietary protein consumption (g) / weight gain (g)) × 1000

Energy utilized kg⁻¹ growth (MJ) = (dietary energy consumption / weight gain (g)) × 1000

Condition factor (K) (%) = (fish weight (g) / (fish length)³ (cm)) × 100

Statistical Analysis

The collected data were subjected to one-way ANOVA and Duncan’s multiple range test (P < 0.05) using the Statgraphics 4.0 statistical software package (Manugistics Incorporated, Rockville, MD, USA) (13).

Results

Following the 12-week growth experiment, mortality ranged from 0% to 8%. Initial and final body weights, as well as FI of each treatment, are shown in Table 3. FI of tilapia fed C, U15, C5, and C15 diets ranged above 3% body weight. On the other hand, for fish fed U5, U10, and C10, average FI was below 3% body weight.
Performance traits of experimental tilapia are presented in Table 3. One of the primary remarkable results was that, except for the U15 diet, no significant difference was noted in the final weight of the fish. The U15 diet produced the poorest final fish weight. Fish fed U5 and C5 diets had similar WG when compared to the control group. Fish fed U10 and C10 diets had 7% and 6% less weight gain, respectively, compared to the control group. WG of fish fed the C15 diet was 90% that of the control group. Tilapia fed the U15 diet had 50% of the WG attained in fish fed the C diet, and the difference was significant (P < 0.05). The FCR (ratio of FI to WG) ranged from 2.1 to 2.7 for all fish in the experiment, except the group fed the U15 diet, in which FCR was drastically raised to 4.2 (P < 0.05). The dietary protein utilized per kg growth varied between 845 (C10) and 1484 g (U15). It was noticed that significantly high protein (P < 0.05) was utilized per kg growth in the groups fed with the highest algae meal concentration (U15 and C15). Fish fed with the U10 and C10 diets utilized the least dietary protein per kg growth. Dietary energy utilized per kg growth was between 40.1 MJ (C10) and 64.4 MJ (U15), and obviously reflected the pattern of those dietary proteins utilized per kg growth. Apparent net protein utilization (ANPU) exceeded 50% for the groups fed U10 (59%), C5 (55.6%), and C10 (50.3%) diets. The lowest ANPU was recorded for the groups fed the U15 (39.0%) and C15 (41.3%) diets. Apparent net energy utilization (ANEU) was in accordance with the behavior of the ANPU parameter in that the ANEU of the U5, U10, C5, and C10 groups showed the highest values (66.5%, 67.4%, 65.3%, and 66.3%, respectively), which was different than that of fish fed the U15 diet (37.8%). Whole body compositions of the fish at the beginning and end of the experiment are presented in Table 4. Final crude protein content of the fish at the end of all treatments dropped significantly (P < 0.05) compared to the initial measurements; however, there were no significant differences (P > 0.05) in the final crude protein content among the treatments. The lipid content of the fish at the end of all treatments was much higher than at the onset (P < 0.05). Carcass lipid levels tended to decrease (P > 0.05) with increasing levels of Ulva meal; however, an increase in carcass lipid level was observed with increasing levels of Cystoseira meal (P < 0.05). A significant (P < 0.05) drop in ash content was marked in all fish at the experiment’s end compared to initial measurements, though among the experimental treatments the ash content was not significantly different (P > 0.05).

### Table 3. Growth, feed, and nutrient utilization of *O. niloticus* (L.) fed the experimental diets.

<table>
<thead>
<tr>
<th>Diet</th>
<th>C</th>
<th>U5</th>
<th>U10</th>
<th>U15</th>
<th>C5</th>
<th>C10</th>
<th>C15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survival (%)</td>
<td>100 ± 0.0</td>
<td>95.2 ± 4.75</td>
<td>92.9 ± 7.12</td>
<td>92.5 ± 7.49</td>
<td>91.9 ± 4.40</td>
<td>94.2 ± 5.77</td>
<td>92.0 ± 8.05</td>
</tr>
<tr>
<td>Initial Mean Weight (g)</td>
<td>4.6 ± 0.13</td>
<td>4.5 ± 0.08</td>
<td>4.5 ± 0.06</td>
<td>4.3 ± 0.04</td>
<td>4.5 ± 0.05</td>
<td>4.5 ± 0.03</td>
<td>4.5 ± 0.04</td>
</tr>
<tr>
<td>Final Mean Weight (g)</td>
<td>11.7 ± 0.80b</td>
<td>11.7 ± 0.40b</td>
<td>11.1 ± 0.70b</td>
<td>7.9 ± 0.24a</td>
<td>12.3 ± 0.43b</td>
<td>11.2 ± 0.19a</td>
<td>10.9 ± 0.47b</td>
</tr>
<tr>
<td>Feed Intake (%)</td>
<td>3.3 ± 0.15</td>
<td>2.7 ± 0.50</td>
<td>2.5 ± 0.29</td>
<td>3.2 ± 0.12</td>
<td>3.2 ± 0.62</td>
<td>2.8 ± 0.33</td>
<td>3.2 ± 0.21</td>
</tr>
<tr>
<td>Weight Gain (%)</td>
<td>151 ± 1.54a</td>
<td>150 ± 1.03</td>
<td>136 ± 2.58b</td>
<td>78 ± 1.60a</td>
<td>156 ± 1.40b</td>
<td>144 ± 0.50b</td>
<td>126 ± 1.78b</td>
</tr>
<tr>
<td>Feed Conversion Ratio</td>
<td>2.7 ± 0.15a</td>
<td>2.1 ± 0.46a</td>
<td>2.1 ± 0.35b</td>
<td>4.2 ± 0.12a</td>
<td>2.3 ± 0.39b</td>
<td>2.2 ± 0.24a</td>
<td>2.7 ± 0.05b</td>
</tr>
<tr>
<td>SGR (%)</td>
<td>1.1 ± 0.05a</td>
<td>1.1 ± 0.03b</td>
<td>1.0 ± 0.07b</td>
<td>0.7 ± 0.03a</td>
<td>1.2 ± 0.04b</td>
<td>1.1 ± 0.01b</td>
<td>1.0 ± 0.05b</td>
</tr>
<tr>
<td>ANPU (%)</td>
<td>53.9 ± 3.50</td>
<td>66.5 ± 13.88</td>
<td>67.4 ± 15.71</td>
<td>37.8 ± 3.04</td>
<td>65.3 ± 14.75</td>
<td>66.3 ± 11.23</td>
<td>58.2 ± 3.44</td>
</tr>
<tr>
<td>Dietary Protein utilized per kg growth (g)</td>
<td>944 ± 115a</td>
<td>984 ± 206a</td>
<td>905 ± 199a</td>
<td>1485 ± 131b</td>
<td>1052 ± 211a</td>
<td>845 ± 241a</td>
<td>1208 ± 102b</td>
</tr>
<tr>
<td>Dietary Energy utilized per kg growth (MJ)</td>
<td>43.2 ± 5.06a</td>
<td>45.9 ± 11.23a</td>
<td>43.7 ± 9.99b</td>
<td>64.4 ± 7.67a</td>
<td>49.0 ± 9.89a</td>
<td>40.1 ± 11.77a</td>
<td>58.5 ± 5.11b</td>
</tr>
<tr>
<td>Condition factor</td>
<td>1.6 ± 0.04</td>
<td>1.6 ± 0.15</td>
<td>1.5 ± 0.16</td>
<td>1.4 ± 0.09</td>
<td>1.5 ± 0.07</td>
<td>1.5 ± 0.20</td>
<td>1.5 ± 0.05</td>
</tr>
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</table>

* Values in each row allocated common superscripts or without superscripts are not significantly different from each other (P > 0.05).
Discussion

Based on the results of this study, it was concluded that Cystoseira and Ulva meals could be incorporated into tilapia diets. Fish mortality was less than 10% for the duration of the experiment. The observed fish loss was probably not due to dietary inclusion of macroalgae meals, but rather due to initial handling during weighing and daily routine management of the experimental system. Fish attained the near-maximum growth potential by regulating feed intake, since they were fed to satiation throughout the trial. In this study, growth performance of fish tended to decrease with an increase in both algae meal concentrations. This tendency was remarkable as Ulva supplementation increased. The growth rates recorded in this experiment for groups fed the diets supplemented with various levels of Ulva meal were in accordance with the findings of Mensi et al. (14), who fed Oreochromis niloticus fry a 30% dietary protein containing 9% and 18% Ulva meal for 45 days. A study on the utilization of filamentous green algae (Cladophora glomerata) as a protein source in pelleted feeds for tilapia (Oreochromis niloticus) reported the highest growth rates for fish fed the diet containing the least algae meal (1). These results were similar to previous studies of black sea bream (Acanthopagrus schlegeli) (10), tilapia (Oreochromis niloticus) (14), and snakehead (Channa striatus) (3). Wassef et al. (11) contrarily indicated that the best weight gain in striped mullet (Mugil cephalus) was obtained with a diet including 20% Ulva, whereas Kissil et al. (15) observed no effect of Ulva inclusion on fish growth in the gilthead sea bream, Sparus aurata. Therefore, 5% to 10% dietary Ulva supplementation enhances the growth rate, but the determination of optimal supplementation level depends on both the Ulva and fish species. Moreover, fish size should not be ignored. In tilapia feeds, not only macroalgae, but also microalgae and aquatic plants have been utilized. For instance, El-Sayed (16) replaced fish meal with Azolla pinnata for Nile tilapia fingerlings and adults. They noted that fish fed A. pinnata showed extremely poor performance, even at the lowest inclusion level (25%). In contrast, Naegel (17) found that up to 30% of the fish meal-based diet fed to tilapia could be successfully replaced with dried Azolla meal. From the view of utilization of alternative algae species in aquafeed, our concern was to evaluate Cystoseira barbata for the first time as a feed additive for Nile tilapia. The growth rates did not drop sharply with increasing Cystoseira concentration, as was the case for fish fed increasing concentrations of Ulva meal. This enables a higher concentration of Cystoseira meal (5%-15%) in a diet. In this study, the only poor performance in FCR was recorded by increasing Ulva meal to 15%. Mensi et al. (14) reported that 9% of dietary Ulva supplementation improved feed efficiency in Nile tilapia when compared to supplementation with 18% Ulva meal. Mustafa et al. (18) reported a considerable improvement in feed utilization in young red sea bream when fed moist pellets supplemented with Ascophyllum, Porphyra, and Ulva meal at a level of 5%. Feeding diets supplemented with Ulva meal to black sea bream favorably influenced feed utilization, but this parameter dropped as the inclusion level increased to more than 10% (19), whereas a 20% dietary supplementation of Ulva meal resulted in higher feed utilization for striped mullet (11). Studies conducted by Takeuchi et al. (20) presented different FCR values for similarly sized juvenile tilapia fed different levels of Spirulina in a couple of experiments. It could be mentioned that one of the factors affecting feed utilization is the form of diet; whether the algae that is supplemented is freshly harvested or if dry commercial

Table 4. Body composition of O. niloticus (L.) fed the experimental diets.

<table>
<thead>
<tr>
<th></th>
<th>Initial</th>
<th>C</th>
<th>U5</th>
<th>U10</th>
<th>U15</th>
<th>C5</th>
<th>C10</th>
<th>C15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (%)</td>
<td>70.1 ± 1.57</td>
<td>70.3 ± 1.71</td>
<td>72.0 ± 1.49</td>
<td>68.6 ± 3.05</td>
<td>71.7 ± 2.72</td>
<td>73.9 ± 0.89</td>
<td>72.7 ± 1.53</td>
<td>71.7 ± 1.68</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>19.3 ± 1.60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.3 ± 0.96&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.4 ± 0.65&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.2 ± 0.82&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.5 ± 1.63&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.1 ± 0.60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.2 ± 1.64&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.9 ± 1.61&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lipid (%)</td>
<td>4.4 ± 1.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.9 ± 0.52&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>8.9 ± 0.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.8 ± 0.79&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>7.8 ± 1.58&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.0 ± 0.27&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.2 ± 0.92&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.7 ± 0.83&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>4.5 ± 0.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.6 ± 0.55&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>3.4 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.7 ± 0.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.4 ± 0.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.4 ± 0.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.3 ± 0.51&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.3 ± 0.31&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

* Values in each row allocated common superscripts or without superscripts are not significantly different from each other (P > 0.05).
algae meal powder is made into pellets. Processing the feed ingredients may influence the chemical composition of the diets, which may affect feed utilization. ANPU and ANEU observations of the present study indicated that the inclusion of about 5%-10% of both algae species meals promoted nutrient and energy utilization, compared to the control diet (C). The improvement was possibly the result of improved efficiency of absorption and assimilation of dietary protein due to algae meal supplementation. In our study, protein and energy utilization of fish fed up to 10% Ulva meal and 15% Cystoseira meal were high. Replacing fish meal with a high level of Ulva meal may depress digestible protein in the diet. It may be concluded that optimum growth, and nutrient as well as energy utilization, are achieved by adjusting dietary algae meal to a certain extent according to the dietary protein requirements of the fish under examination. Concerning protein sparing, fish fed the U10 and C10 diets spared considerable protein for growth when compared to the fish fed the U15 and C15 diets. Fish fed U10 utilized approximately 64% less dietary protein and 47% less dietary energy per kg growth than fish fed the U15 diet. A similar pattern was observed for fish fed C10, in which 43% less dietary protein and 46% less dietary energy was utilized per kg growth than in fish fed the C15 diet. In the present study, moisture and ash content of fish were not significantly affected by the dietary supplementation of Ulva and Cystoseira meal, while their protein content decreased in comparison to the experiment’s onset. Despite a marked increase in body lipid with an increase in the level of Cystoseira meal, the reverse was observed when Ulva meal supplementation was increased. It might be that increasing dietary Cystoseira meal improved the absorption and assimilation of dietary protein, and, as a result, spared dietary energy was converted into lipid reserves. Earlier studies state that macro- and microalgae supplementation of diets influences lipid accumulation and composition of fish (2,4); however, based on our results, we cannot conclude that higher concentrations of Ulva cause a decrease in body lipid since it only demonstrated a tendency. In conclusion, the current investigation revealed that the optimum dietary supplementation level of Ulva meal and Cystoseira meal on body composition could be up to 10% and 15%, respectively, in juvenile Oreochromis niloticus. With regard to the practical use of Ulva and Cystoseira meal in Nile tilapia feed, further studies are required to clarify the mechanism or mechanisms of effect of dietary Ulva and Cystoseira meal. Future work should focus on a few points, such as establishing the digestibility coefficients of different nutrient classes in macroalgae sources prior to their inclusion in formulated diets, and the effects of various algae meal supplementation on long-term feeding and their influence on flesh quality in fresh water and marine fish.

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References


