Effects of Gracilaria, Enteromorpha prolifera, Algae Residue, and Fungal Residue as Fish Meal Replacements on Growth Performance, Serum and Liver Biochemical Indices, Body Composition, and Intestinal Histology in Juvenile Turbot (Scophthalmus maximus) Postprint

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Abstract

This experiment aimed to investigate the effects of replacing dietary fish meal with Gracilaria, Enteromorpha, algae residue, and fungal residue on growth performance, serum and liver biochemical indices, body composition, and intestinal histology of juvenile turbot. Five isonitrogenous and isolipidic diets were formulated: a basal diet containing 60% fish meal (fish meal group, as control), and four experimental diets in which 35% of the fish meal in the basal diet was replaced by a combination of 10% Gracilaria, Enteromorpha, algae residue, or fungal residue with plant proteins (wheat gluten meal, corn gluten meal, and soybean meal). The diets were fed to juvenile turbot with initial body weight of (16.00$±$0.11) g for 77 days. The results showed: 1) The weight gain rate and specific growth rate of Gracilaria, algae residue, and fungal residue groups showed no significant difference compared with the fish meal group ($P>0.05$), while the Enteromorpha group was significantly lower than the fish meal group ($P<0.05$); feed efficiency of Gracilaria and fish meal groups showed no significant difference ($P>0.05$), and both were significantly higher than Enteromorpha, algae residue, and fungal residue groups ($P<0.05$); protein retention rate of the fish meal group was significantly higher than Enteromorpha, algae residue, and fungal residue groups ($P<0.05$), with no significant difference from the Gracilaria group ($P>0.05$). 2) The crude protein content in fish body of Gracilaria and Enteromorpha groups was significantly lower than the fish meal group ($P<0.05$), while the crude lipid content in fish body of algae residue and fungal residue groups was significantly higher than the fish meal group ($P<0.05$); the histidine and taurine contents in muscle of

Gracilaria, Enteromorpha, algae residue, and fungal residue groups were significantly lower than the fish meal group (P<0.05), and the lysine content in muscle of Enteromorpha and algae residue groups was significantly lower than the fish meal group (P<0.05); there were no significant differences in serum alanine aminotransferase, aspartate aminotransferase activities, and liver aspartate aminotransferase activity among all groups (P>0.05), while liver alanine aminotransferase activity of the fish meal group was significantly higher than Gracilaria and fungal residue groups (P<0.05), with no significant difference from Enteromorpha and algae residue groups (P>0.05). 3) The foregut and midgut fold heights of the fungal residue group were significantly higher than the fish meal group (P<0.05), and the foregut fold height of the algae residue group was significantly higher than the fish meal group (P<0.05), while there were no significant differences in foregut and midgut microvillus heights among all groups (P>0.05). In summary, replacing 35% of dietary fish meal with a combination of 10% Gracilaria, algae residue, and fungal residue with plant proteins had no adverse effects on growth performance of juvenile turbot, and algae residue and fungal residue had improving effects on intestinal histology of juvenile turbot.

Full Text

Effects of Gracilaria verrucosa, Enteromorpha prolifera, Algae Residue and Fungi Residue on Growth Performance, Serum and Liver Biochemical Indices, Body Composition and Intestinal Histological Morphology of Juvenile Turbot (Scophthalmus maximus L.)

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Abstract: This experiment investigated the effects of replacing dietary fish meal with Gracilaria verrucosa, Enteromorpha prolifera, algae residue and fungi residue on growth performance, serum and liver biochemical indices, body composition and intestinal histological morphology of juvenile turbot (*Scophthalmus maximus* L.). Five isonitrogenous and isolipidic diets were formulated: a basal diet containing 60% fish meal served as the control (fish meal group), while four experimental diets replaced 35% of the fish meal in the basal diet with a combination of 10% Gracilaria verrucosa, Enteromorpha prolifera, algae residue (waste from alginate extraction from kelp) or fungi residue (waste from *Aspergillus terreus* fermentation for itaconic acid production) plus plant proteins (wheat gluten, corn gluten meal and soybean meal). The diets were fed to juvenile turbot with an initial body weight of (16.00$±$0.11) g for 77 days. The results showed: 1) Weight gain rate and specific growth rate of the Gracilaria, algae residue and fungi residue groups were not significantly different from the fish meal group ($P>0.05$), while the Enteromorpha group was significantly lower ($P<0.05$). Feed efficiency of the Gracilaria group did not differ significantly from the fish meal group, and both were significantly higher than the Enteromorpha, algae residue and fungi residue groups ($P<0.05$). Protein productive value of the fish meal group was significantly higher than the Enteromorpha, algae residue and fungi residue groups ($P<0.05$), but not significantly different from the Gracilaria group ($P>0.05$). 2) Crude protein content in fish body was significantly lower in the Gracilaria and Enteromorpha groups than the fish meal group ($P<0.05$), while crude lipid content was significantly higher in the algae residue and fungi residue groups ($P<0.05$). Muscle histidine and taurine contents were significantly lower in all experimental groups compared to the fish meal group ($P<0.05$), and muscle lysine content was significantly lower in the Enteromorpha and algae residue groups ($P<0.05$). No significant differences were observed in serum glutamic-pyruvic transaminase, glutamic-oxaloacetic transaminase activities or liver glutamic-oxaloacetic transaminase activity among groups ($P>0.05$), though liver glutamic-pyruvic transaminase activity in the fish meal group was significantly higher than in the Gracilaria and fungi residue groups ($P<0.05$). 3) Foregut and midgut mucosal fold heights in the fungi residue group were significantly higher than in the fish meal group ($P<0.05$), and foregut mucosal fold height in the algae residue group was also significantly higher than in the fish meal group ($P<0.05$). No significant differences were found in foregut and midgut microvillus heights among groups ($P>0.05$). In conclusion, replacing 35% of fish meal with 10% Gracilaria verrucosa, algae residue or fungi residue combined with plant proteins had no adverse effects on growth performance of juvenile turbot, and algae residue and fungi residue improved intestinal histological structure.

Keywords: juvenile turbot; seaweed; growth performance; body composition; intestinal histological structure

Introduction

Global aquaculture production has increased rapidly in recent years, and the expansion of farming scale has increased demand for aquafeeds. Fish meal is the primary protein source in aquafeeds, but due to overfishing, environmental pollution and adverse climate events such as El Niño, wild fish meal resources are decreasing while prices continue to rise [1]. Therefore, reducing fish meal usage and developing suitable protein alternatives is critically important for the aquafeed industry.

Plant proteins have long been used as fish meal alternatives due to their wide availability, stable supply and low cost. Although numerous studies have investigated traditional plant proteins in aquafeeds [2-5], they cannot completely replace fish meal because they contain antinutritional factors [6] and lack certain essential amino acids and growth-promoting components required by fish. Seaweeds are autotrophic marine organisms containing chlorophyll. Compared with terrestrial plants, seaweeds are rich in minerals, vitamins, seaweed polysaccharides, unsaturated fatty acids, free amino acids and feeding stimulants that can compensate for nutritional deficiencies caused by large-scale replacement of fish meal with terrestrial plant proteins [7-8]. China has vast sea areas and abundant seaweed resources that remain underutilized. If seaweed protein resources can be fully exploited, it would be significant for solving protein source issues in aquafeeds. Therefore, this study used two common low-value seaweeds (Gracilaria verrucosa and Enteromorpha prolifera) and two industrial by-products (algae residue from alginate extraction from kelp and fungi residue from *Aspergillus terreus* fermentation for itaconic acid production) combined with plant proteins to replace partial fish meal in juvenile turbot diets, investigating effects on growth performance, serum and liver biochemical indices, body composition and intestinal morphology to provide a theoretical basis for application of seaweeds and industrial by-products in turbot feeds.

1.1 Experimental Diets

Five isonitrogenous and isolipidic experimental diets were formulated. A basal diet containing 60% fish meal as the main protein source and fish oil as the lipid source served as the control (fish meal group). Four other diets replaced 35% of fish meal in the basal diet with combinations of 10% Gracilaria verrucosa, Enteromorpha prolifera, algae residue (waste from alginate extraction from kelp) or fungi residue (waste from *Aspergillus terreus* fermentation for itaconic acid production) plus plant proteins (wheat gluten, corn gluten meal and soybean meal). After analyzing conventional nutritional components, all ingredients were ground to pass through an 80-mesh sieve, weighed according to formulation, and mixed stepwise. Fish oil was then added and mixed, followed by 30% water to form a uniform dough. The mixture was pelleted into 2 mm diameter particles using a pellet mill, dried at 55°C for 12 hours with forced air, and stored at -20°C. The proximate nutritional components of Gracilaria verrucosa, Enteromorpha prolifera, algae residue and fungi residue are shown in Table 1 . Diet composition and nutrient levels are presented in Table 2 , and amino acid composition of experimental diets is shown in Table 3 .

Table 1 Proximate nutritional components of Gracilaria verrucosa, Enteromorpha prolifera, algae residue and fungi residue (DM basis)

Items	Gracilaria verrucosa	Enteromorpha prolifera	Algae residue	Fungi residue
Crude protein				

Items	Gracilaria verrucosa	Enteromorpha prolifera	Algae residue	Fungi residue
Crude lipid				
Ash				

Table 2 Composition and nutrient levels of experimental diets (DM basis)

Items	Fish meal	Gracilaria verrucosa	Enteromorpha prolifera	Algae residue	Fungi residue
Ingredients					
Fish meal					
Gracilaria verrucosa					
Enteromorpha prolifera					
Algae residue					
Fungi residue					
Wheat gluten					
Corn gluten meal					
Soybean meal					
Wheat meal					
Phospholipid					
Choline					
Fish oil					
$Ca(H_2PO_4)_2$					
Vitamin premix[1]					

Items	Fish meal	Gracilaria verrucosa	Enteromorpha prolifera	Algae residue	Fungi residue
Mineral premix[2]					
Total					
Nutrient levels					
Crude protein					
Crude lipid					
Ash					

[1] Vitamin premix contained per kg: VA 375,000 IU, VD_3 75,000 IU, VE 3,000 mg, VK_3 900 mg, VB_1 600 mg, VB_2 600 mg, VB_6 600 mg, VB_{12} 3.7 mg, D-calcium pantothenate 2,400 mg, niacinamide 4,500 mg, folic acid 185 mg, D-biotin 7.5 mg, inositol 3,000 mg, VC 10,500 mg.

[2] Mineral premix contained per kg: Zn 1,750 mg, Mn 1,050 mg, Cu 410 mg, Fe 1,150 mg, Co 60 mg, I 50 mg, Se 15 mg.

Table 3 Amino acid composition of experimental diets (DM basis)

Amino acids	Fish meal	Gracilaria verrucosa	Enteromorpha prolifera	Algae residue	Fungi residue
Essential amino acids (EAA)					
Thr					
Val					
Met					
Ile					
Leu					
Phe					
Lys					
His					
Arg					
ΣEAA					

Amino acids	Fish meal	Gracilaria verrucosa	Enteromorpha prolifera	Algae residue	Fungi residue
Non-essential amino acids (NEAA)					
Asp					
Ser					
Glu					
Gly					
Ala					
Cys					
Tyr					
Tau					
ΣNEAA					
ΣEAA/ΣNEAA					

1.2 Feeding Management

The feeding trial was conducted at Yantian Yuan Aquatic Co., Ltd. in Yantai, Shandong Province. Juvenile turbot were obtained from the same company and acclimated to the experimental conditions and diet for one week using the control diet before the trial began. The experiment used natural lighting and a flow-through culture system with deep well seawater. Water temperature was maintained at 12-14°C, dissolved oxygen concentration at approximately 5.5 mg/L, salinity at approximately 35‰, and pH at 7.5-8.0.

Prior to the experiment, fish were fasted for 24 hours. Healthy, uniformly sized juvenile turbot with an initial body weight of (16.00$±$0.11) g were randomly selected and stocked into 15 plastic tanks (150 L capacity) at 25 fish per tank. The 15 tanks were randomly divided into 5 groups with 3 replicate tanks per group, and each group was randomly assigned one experimental diet. During the 77-day trial period (September to November 2016), fish were fed to apparent satiation twice daily (morning and evening). Uneaten feed was collected 0.5 hours after feeding and counted, and residual feed mass was calculated based on the average weight per 100 feed pellets.

1.3 Sample Collection and Analysis

At the start of the experiment, 20 juvenile turbot were randomly sampled as initial fish for proximate composition analysis. At the end of the trial, fish were fasted for 24 hours, then counted and weighed per tank. Three fish per tank were randomly selected for blood collection via caudal vein using 1% heparin sodium as anticoagulant. Blood was left at 4°C for 4 hours, then centrifuged at 3,500 r/min for 10 minutes to obtain serum, which was stored in liquid nitrogen. After blood collection, fish were weighed and measured for body length, then dissected to separate viscera and liver for calculation of condition factor, hepatosomatic index and viserosomatic index. Livers were stored in liquid nitrogen. Skinless dorsal muscle samples from the same location were collected and stored in liquid nitrogen for amino acid analysis. Three additional fish per tank were randomly sampled for whole-body composition analysis. One fish per tank was randomly selected for intestinal histology; the foregut and midgut were fixed in Davidson' s solution (330 mL 95% ethanol, 220 mL formaldehyde, 115 mL glacial acetic acid, 335 mL purified water) for 24 hours, then transferred to 70% ethanol for storage.

Proximate composition analysis of feeds and fish followed AOAC (1995) methods: moisture by drying at 105°C to constant weight, crude protein by Kjeldahl method (VELP Kjeldahl analyzer, UDK-142 Automatic Distillation Unit, Italy), crude lipid by Soxhlet extraction with petroleum ether (SOXTEC 2050 FOSS, Sweden), and ash by muffle furnace incineration at 550°C for 6 hours. Serum triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), malondialdehyde (MDA) and total bile acid (TBA) contents, as well as superoxide dismutase (SOD), acid phosphatase (ACP), alkaline phosphatase (AKP), glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) activities in serum, and GOT and GPT activities in liver were measured using assay kits from Nanjing Jiancheng Bioengineering Institute.

Amino acid composition in feeds and muscle was determined according to GB/T 18246-2000 using an L-8900 automatic amino acid analyzer (Hitachi, Japan). Nine essential amino acids, seven non-essential amino acids and taurine were measured; tryptophan was not analyzed due to destruction by acid hydrolysis.

Intestinal histological analysis: Paraffin sections were prepared using paraffin embedding and hematoxylin-eosin (HE) staining, observed and photographed under a microscope (80i, Nikon, Japan). Mucosal fold height, enterocyte height and microvillus height were measured using Photoshop CC 2017 software.

1.4 Calculation Formulas

- Survival rate (SR, %) = 100 × final fish number / initial fish number
- Feed intake (FI, %/d) = 100 × dry feed intake / [trial days × (initial body weight + final body weight) / 2]
- Weight gain rate (WGR, %) = 100 × (final body weight - initial body weight) / initial body weight
- Specific growth rate (SGR, %/d) = 100 × (ln final body weight - ln initial body weight) / trial days
- Feed efficiency ratio (FER) = (final body weight - initial body weight) / dry feed intake
- Protein productive value (PPV, %) = 100 × fish protein deposition / total feed protein intake

- Protein efficiency ratio (PER) = (final body weight - initial body weight) / total feed protein intake
- Hepatosomatic index (HSI, %) = 100 × liver weight / body weight
- Viscerosomatic index (VSI, %) = 100 × viscera weight / body weight
- Condition factor (CF, g/cm^3) = 100 × body weight / body $length^3$ (body weight in g, body length in cm)

1.5 Data Analysis

Experimental data were processed using SPSS 17.0 software. One-way ANOVA was used for variance analysis, and Duncan' s multiple comparison test was applied when significant differences were detected ($P < 0.05$). Results are expressed as mean ± standard error (SE).

2.1 Effects of Fish Meal Replacement on Growth Performance

Effects of Gracilaria verrucosa, Enteromorpha prolifera, algae residue and fungi residue on growth performance of juvenile turbot are shown in Table 4 . Survival rates ranged from 92% to 96% with no significant differences among groups ($P>0.05$). Weight gain rate and specific growth rate of the Gracilaria, algae residue and fungi residue groups were not significantly different from the fish meal group ($P>0.05$), while the Enteromorpha group was significantly lower ($P<0.05$). The fungi residue and Enteromorpha groups were significantly lower than the algae residue group ($P<0.05$), while the Gracilaria group showed no significant differences with the other three groups ($P>0.05$). Feed intake of the Enteromorpha and algae residue groups was significantly higher than the fish meal and Gracilaria groups ($P<0.05$), and the fungi residue group was significantly higher than the Gracilaria group ($P<0.05$), though neither differed significantly from the fish meal group ($P>0.05$). Feed efficiency and protein efficiency ratio of the fish meal and Gracilaria groups were not significantly different, but both were significantly higher than the Enteromorpha, algae residue and fungi residue groups ($P<0.05$). Protein productive value of the fish meal and Gracilaria groups was significantly higher than the Enteromorpha and algae residue groups ($P<0.05$), with no significant difference between the Gracilaria and fungi residue groups ($P>0.05$). Viscerosomatic index of the fish meal group was significantly lower than the algae residue group ($P<0.05$) but not significantly different from the Gracilaria, Enteromorpha and fungi residue groups ($P>0.05$). No significant differences were observed in hepatosomatic index or condition factor among groups ($P>0.05$).

Table 4 Effects of fish meal replacement by Gracilaria verrucosa, Enteromorpha prolifera, algae residue and fungi residue on growth performance and physical indicators of juvenile turbot

Items	Fish meal	Gracilaria verrucosa	Enteromorpha prolifera	Algae residue	Fungi residue
SR (%)	96.00$±4.00|94.67±1.33|92.00±2.31|96.00±2.31|94.67±2.67||WGR(±3.47ab|127.79±5.97abc|120.28±3.				

In the same row, values with no letter or the same letter superscripts mean no significant difference (P>0.05), while different letter superscripts mean significant difference (P<0.05). The same as below.

2.2 Effects of Fish Meal Replacement on Serum and Liver Biochemical Indices

Effects of fish meal replacement on serum TG, TC, HDL-C, LDL-C and TBA contents are shown in Table 5 . No significant differences were found in serum TG, LDL-C and TBA contents among groups (P>0.05). Serum TC content in the Enteromorpha group was significantly lower than the algae residue group (P<0.05) but significantly higher than the fish meal and fungi residue groups (P<0.05), with no significant difference from the Gracilaria group (P>0.05). Serum HDL-C content in the Enteromorpha and algae residue groups was significantly higher than the fish meal and fungi residue groups (P<0.05), but not significantly different from the Gracilaria group (P>0.05).

Table 5 Effects of fish meal replacement on serum TG, TC, HDL-C, LDL-C and TBA contents of juvenile turbot

Items	Fish meal	Gracilaria verrucosa	Enteromorpha prolifera	Algae residue	Fungi residue
TG (mmol/L)	2.71$±0.94|1.63±0.75|1.64±0.86|3.38±1.42|4.22±2.40||TC(mmol/L)|2.70±0.03c|2.95±0.06bc|3.16±0.1 C(mmol/L)|2.15±0.16b|2.63±0.08ab|2.89±0.24a|2.81±0.26a|1.90±0.20c||LDL−C(mmol/L)|0.38±0.01|0.28±0.04|0.25±0.06|0.43±0.14|0.32±0.07||TBA(μmol/L)|7.71±1.64|5.95±0.17				

Effects of fish meal replacement on GPT and GOT activities in serum and liver are shown in Table 6 . No significant differences were observed in serum GPT and GOT activities or liver GOT activity among groups (P>0.05). Liver GPT activity in the fish meal group was significantly higher than in the fungi residue and Gracilaria groups (P<0.05), but not significantly different from the Enteromorpha and algae residue groups (P>0.05).

Table 6 Effects of fish meal replacement on GPT and GOT activities in serum and liver of juvenile turbot

<table>
<tr><th>Items</th><th>Fish meal</th><th>Gracilaria verrucosa</th><th>Enteromorpha prolifera</th><th>Algae residue</th><th>Fungi residue</th></tr>
<tr><td>Serum</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>GPT (U/L)</td><td colspan="5">7.31$±3.99|6.67±1.48|6.46±4.65|12.32±5.01|6.93±2.84||<i>GOT</i>(<i>U/L</i>)|8.38±2.85|6.30±1.07|7.20±5.16|12.
∗<i>Liver</i>∗
∗||||||<i>GPT</i>(<i>U/gprot</i>)|89.12±4.02<i>a</i>|67.16±0.51<i>c</i>|85.92±3.22<i>ab</i>|86.18±2.53<i>ab</i>|78.23±4.03<i>b</i>||<i>GOT</i>(<i>U/gpro</i></td></tr>
</table>

Effects of fish meal replacement on serum non-specific immune and antioxidant indices are shown in Table 7 . Serum ACP activity in the Gracilaria, Enteromorpha, algae residue and fungi residue groups was not significantly different from the fish meal group (P>0.05), though the algae residue group was significantly higher than the Enteromorpha group (P<0.05). No significant differences were found in serum AKP activity or MDA content among groups (P>0.05). Serum SOD activity in the Gracilaria, Enteromorpha, algae residue and fungi residue groups was not significantly different from the fish meal group (P>0.05), but the Gracilaria and algae residue groups were significantly higher than the Enteromorpha and fungi residue groups (P<0.05).

Table 7 Effects of fish meal replacement on serum non-specific immune and antioxidant indices of juvenile turbot

<table>
<tr><th>Items</th><th>Fish meal</th><th>Gracilaria verrucosa</th><th>Enteromorpha prolifera</th><th>Algae residue</th><th>Fungi residue</th></tr>
<tr><td>ACP (King’s unit/dL)</td><td colspan="5">1.71$±0.37<i>ab</i>|1.92±0.25<i>ab</i>|1.27±0.23<i>b</i>|2.67±0.45<i>a</i>|1.64±0.16<i>ab</i>||<i>AKP</i>(<i>King′sunit/dL</i>)|2.04±0.62|1.35±</td></tr>
</table>

2.3 Effects of Fish Meal Replacement on Body Composition and Muscle Amino Acid Composition

Effects of fish meal replacement on body composition are shown in Table 8 . No significant differences were observed in moisture or ash content among groups (P>0.05). Crude protein content in the fish meal group was significantly higher than in the Gracilaria and Enteromorpha groups (P<0.05), but not significantly different from the algae residue and fungi residue groups (P>0.05). Crude lipid content in the algae residue and fungi residue groups was significantly higher than in the fish meal group (P<0.05), but not significantly different from the Gracilaria and Enteromorpha groups (P>0.05).

Table 8 Effects of fish meal replacement on body composition of juvenile turbot (%)

<table>
<tr><th>Items</th><th>Fish meal</th><th>Gracilaria verrucosa</th><th>Enteromorpha prolifera</th><th>Algae residue</th><th>Fungi residue</th></tr>
<tr><td>Moisture</td><td colspan="5">77.51$±0.17|78.39±0.38a|77.57±0.41ab|77.09±0.39b|76.51±0.06b||Crudeprotein|14.93±0.05|14.07±0.2</td></tr>
</table>

Effects of fish meal replacement on muscle amino acid composition are shown in Table 9 . Muscle lysine content in the fish meal group was significantly higher than in the Enteromorpha and algae residue groups (P<0.05), but not significantly different from the Gracilaria and fungi residue groups (P>0.05). Muscle histidine and taurine contents in the fish meal group were significantly higher than all experimental groups (P<0.05). Muscle alanine content in the Gracilaria group was significantly higher than in the fish meal and fungi residue groups (P<0.05), but not significantly different from the Enteromorpha and algae residue groups (P>0.05). No significant differences were found in other muscle amino acids among groups (P>0.05). Total essential amino acid content in muscle of the fish meal group was significantly higher than in the Enteromorpha and algae residue groups (P<0.05), but not significantly different from the Gracilaria and fungi residue groups (P>0.05). No significant differences were observed in total non-essential amino acid content among groups (P>0.05). Total amino acid content in the fish meal group was significantly higher than in the algae residue group (P<0.05), but not significantly different from the Gracilaria, Enteromorpha and fungi residue groups (P>0.05).

Table 9 Effects of fish meal replacement on amino acid composition in muscle of juvenile turbot (DM basis)

<table>
<tr><th>Amino acids</th><th>Fish meal</th><th>Gracilaria verrucosa</th><th>Enteromorpha prolifera</th><th>Algae residue</th><th>Fungi residue</th></tr>
<tr><td>Essential amino acids (EAA)</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Thr</td><td colspan="5">4.07$±0.|3.95±0.10|3.88±0.05|3.88±0.0|3.94±0.0||Val|3.97±0.|3.82±0.06|3.79±0.03|3.84±0.0|3.82± ∗Non−essentialaminoacids(NEAA)∗ ∗||||||Asp|8.98±0.1|8.85±0.27|8.68±0.15|8.63±0.1|8.8±0.09||Ser|3.86±0.|3.7±0.12|3.63±0.08|3.64± ∗Total∗ ∗||||||TAA|82.19±0|80.37±1.14ab|78.96±1.16ab|78.8±1.0|79.64±0.||ΣEAA/ΣNEAA|0.97±0.|0.96±</td></tr>
</table>

2.4 Effects of Fish Meal Replacement on Intestinal Histological Structure

The midgut cross-sectional structure of juvenile turbot in each group is shown in Figure 1 [Figure 1: see original paper]. MF: mucosal fold; EH: enterocyte; MV: microvillus. A, a: fish meal group; B, b: Gracilaria verrucosa group; C, c: Enteromorpha prolifera group; D, d: algae residue group; E, e: fungi residue group. Scale bars for A, B, C, D and E: 400 m; scale bars for a, b, c, d and e: 40 m.

Figure 1 The midgut transection structure of juvenile turbot in each group

Effects of fish meal replacement on intestinal histological structure are shown in Table 10 . Foregut mucosal fold height in the algae residue group was significantly higher than in the fish meal group (P<0.05), significantly lower than in the fungi residue group (P<0.05), and not significantly different from the Gracilaria and Enteromorpha groups (P>0.05). Foregut enterocyte height in the algae residue group was significantly higher than in the fish meal, Gracilaria and Enteromorpha groups (P<0.05), but not significantly different from the fungi residue group (P>0.05). No significant differences were found in foregut microvillus height among groups (P>0.05). Midgut mucosal fold height in the fungi residue group was significantly higher than in the fish meal group (P<0.05), but not significantly different from the Gracilaria, Enteromorpha and algae residue groups (P>0.05). No significant differences were observed in midgut enterocyte height or microvillus height among groups (P>0.05).

Table 10 Effects of fish meal replacement on intestinal histological structure of juvenile turbot

Items	Fish meal	Gracilaria verrucosa	Enteromorpha prolifera	Algae residue	Fungi residue
Foregut					
Mucosal fold height	910.53\$±26.18bc|917.39±47.15bc|801.09±30.0|941.06±32.8|1073.43±55.02a||$Enterocyteheight$|45.09±1 $*Midgut*$ $*$||||||$Mucosalfoldheight$|582.61±33.46ab|585.51±30.19ab|649.28±28.62a|536.96±11.9|759.03±37.8||E				

3.1 Effects on Growth Performance

Numerous studies have reported on seaweed application in aquafeeds, including common species such as *Gracilaria*, *Gelidium*, *Porphyra* (Rhodophyta), *Ulva*, *Enteromorpha* (Chlorophyta), and kelp (Phaeophyta) [9-14]. Many studies have shown that dietary inclusion of small amounts of seaweed (2.5%-10.0%) benefits fish growth by improving growth performance, increasing feed intake and efficiency, and enhancing physiological activity, disease resistance and stress tolerance [9-12].

In this study, when Gracilaria, Enteromorpha and algae residue were combined with plant proteins to replace partial fish meal, the Gracilaria group showed no significant reduction in major essential amino acids compared to the fish meal group (Table 3). In contrast, the Enteromorpha group had substantially lower lysine, methionine and histidine contents, which may explain the inferior growth rate, feed efficiency and protein productive value compared to the Gracilaria group. The algae residue group showed better growth performance than the Enteromorpha group with no significant difference from the fish meal group, despite reduced essential amino acid levels compared to the fish meal diet. This may be attributed to polysaccharides in algae residue, such as fucoidan, laminarin, alginate starch and alginates [15], which promoted growth. This is consistent with Lin et al. [16] who reported that 0.6% kelp polysaccharide improved growth and reduced feed coefficient in pearl gentian grouper. Similar findings were reported by Yang et al. [17] who found that 0.10% fucoidan improved weight gain, specific growth rate and digestive enzyme activities in yellow catfish.

The Enteromorpha and algae residue groups showed significantly higher feed intake than the fish meal and Gracilaria groups, possibly due to feeding stimulants in these ingredients. Studies have shown that seaweeds commonly contain feeding stimulants such as dimethyl-β-propiothetin (DMPT or DMSP) [18-19] that increase feed intake. However, the Gracilaria group did not show increased feed intake, possibly because it contains fewer feeding stimulants. Since these compounds were not measured in this study, the exact reason requires further investigation. Despite lower feed intake, the Gracilaria group showed good growth performance, likely due to both adequate essential amino acids and taurine content. Taurine can regulate digestive enzyme activity, enhance nutrient absorption and utilization, and improve feed efficiency [20]. The Gracilaria diet had higher taurine content than the Enteromorpha and algae residue diets, which may explain why reduced feed intake did not impair growth performance.

Previous studies on plant protein replacement in turbot have shown limited replacement levels. Soy protein concentrate could only replace 17% of fish meal without affecting growth [21]; corn gluten meal could replace 21% without affecting growth performance and feed efficiency [22]; composite plant proteins replacing 20.7% of fish meal significantly reduced growth performance [23]; and enzymatically hydrolyzed animal cartilage protein combined with plant proteins could replace 24% of fish meal without affecting growth and feed intake [24]. Similar results have been reported for other fish species. Wang et al. [25] found soybean meal could replace 16% of fish meal without affecting sea bass growth; Wu et al. [26] found soybean meal replacement of 13.4% did not affect golden perch growth; and Cui et al. [27] found rapeseed meal could replace 13.5% of fish meal without affecting rainbow trout growth performance and feed utilization. In this study, Gracilaria, Enteromorpha, algae residue and fungi residue combined with plant proteins replaced 35% of fish meal, yet the Gracilaria, algae residue and fungi residue groups did not show significantly reduced growth performance. This indicates that compared with traditional terrestrial plant proteins, marine plant protein sources like Gracilaria and algae residue can mitigate growth reduction caused by high-level fish meal replacement, and fungi residue as a fungal protein source contains compounds that promote turbot growth.

3.2 Effects on Lipid Metabolism and Non-Specific Immunity

Serum HDL-C and LDL-C contents reflect high- and low-density lipoprotein levels. Increased HDL transports more cholesterol to the liver, while increased LDL transports more cholesterol and triglycerides to the blood [28], collectively affecting serum TC and TG levels. In this study, serum TG changes were consistent with LDL-C trends. Compared with the fish meal group, the Gracilaria and Enteromorpha groups showed reduced serum LDL-C and TG, while the Enteromorpha and algae residue groups had significantly increased serum HDL-C, indicating that dietary Gracilaria, Enteromorpha and algae residue increased HDL and reduced LDL, benefiting blood lipid health in turbot.

MDA is a terminal product of free radical oxidation that is cytotoxic and can cause cross-linking of macromolecules like proteins and nucleic acids, affecting mitochondrial respiratory chain complexes and key enzyme activities [12]. Serum MDA content reflects lipid peroxidation degree and indirectly indicates cellular oxidative damage [12,29]. SOD plays a crucial role in maintaining oxidative-antioxidant balance by scavenging superoxide anion radicals and protecting cells from damage. In this study, no significant differences were found in serum MDA content or SOD activity among groups, indicating that fish meal replacement with Gracilaria, Enteromorpha, algae residue and fungi residue did not adversely affect antioxidant capacity.

Changes in serum GPT and GOT activities are primary indicators of liver damage. These enzymes mainly exist in the liver, with low serum activity under normal conditions. When liver tissue is damaged, increased cell membrane permeability releases GPT and GOT into the blood, elevating serum activity [30]. In this study, no significant differences in serum GPT or GOT activities were observed, indicating that fish meal replacement did not cause liver damage. Amino acids are metabolized mainly through transamination and deamination, with fish primarily using combined deamination to meet physiological needs. GPT and GOT are key enzymes in fish amino acid metabolism, and their liver activity reflects metabolic intensity and liver function [30]. The significantly lower liver GPT activity in the Gracilaria and fungi residue groups suggests slower amino acid metabolism, possibly related to differences in dietary amino acid composition and absorption rates affecting amino acid metabolism and liver GPT activity.

Aquatic animals lack secondary antibody responses in specific immunity mechanisms, making non-specific immunity critically important for defense [30]. Serum ACP and AKP are important indicators for evaluating non-specific immunity in fish, promoting disease resistance and stress tolerance [30-32]. ACP, a lysosomal marker enzyme, participates in phosphate metabolism regulation, signal transduction and energy conversion, while AKP directly participates in phosphate group transfer and metabolism [30]. In this study, serum ACP and AKP activities in experimental groups were not significantly different from the fish meal group, indicating that fish meal replacement did not adversely affect non-specific immunity. Moreover, the algae residue group showed higher ACP and AKP activities than the fish meal group, possibly due to polysaccharides and oligosaccharides enhancing non-specific immunity. Studies have shown that fucoidan can improve mouse immunity and antiviral capacity [33-34], and alginate oligosaccharides can enhance non-specific immunity in turbot [32].

3.3 Effects on Body Composition and Muscle Amino Acid Composition

Differences in body composition among groups may be related to dietary taurine content. Zhou et al. [35] found that taurine increased whole-body protein deposition and reduced lipid deposition in orange-spotted grouper. Zhang et al. [36] found that taurine supplementation in high plant protein diets reduced lipid deposition in turbot. Qi [37] reported that taurine increased protein deposition and reduced lipid deposition in 6.3 g and 48.0 g turbot, but increased both in 165.9 g fish. Taurine regulates protein deposition by modulating secretion of protein synthesis-related hormones [35]; studies have shown that taurine promotes thyroid hormone secretion in carp [38], which accelerates tissue differentiation, growth and protein/enzyme synthesis. Taurine regulates lipid deposition by conjugating bile acids to stimulate secretion, increasing fatty acid oxidation and energy expenditure [39]. In this study, the fish meal group had higher dietary and muscle taurine content and higher serum total bile acid content, consistent with these findings and possibly explaining the higher crude protein and lower crude lipid content.

Muscle amino acid composition analysis revealed that fish meal replacement affected muscle amino acid profiles, primarily due to dietary amino acid composition. Studies have shown that tissue essential amino acid composition is significantly correlated with dietary amino acid composition [40].

3.4 Effects on Intestinal Histological Structure

Mucosal fold height, enterocyte height and microvillus height are important indicators for evaluating intestinal morphological changes. Intestinal fold and microvillus heights expand surface area and determine nutrient absorption capacity. In this study, fish meal replacement with Gracilaria, Enteromorpha, algae residue and fungi residue promoted intestinal development, particularly in the algae residue and fungi residue groups, which showed excellent intestinal development. This may be related to carbohydrates in these ingredients. Algae residue contains kelp polysaccharides and oligosaccharides like mannose, while fungi residue is a by-product of *Aspergillus terreus* fermentation containing fungal cell wall polysaccharides like chitin, whose derivative chitosan can be hydrolyzed to chitooligosaccharides. Studies have shown that kelp polysaccharides increased intestinal villus length in orange-spotted grouper [41]; Pan [42] found that chitooligosaccharides increased intestinal fold and microvillus heights in turbot; Tian et al. [43] found that chitooligosaccharides promoted intestinal development in GIFT tilapia; and Yu et al. [44] reported that mannan oligosaccharides increased intestinal fold height and promoted microvillus development in half-smooth tongue sole. Polysaccharides and oligosaccharides in algae residue and fungi residue may be responsible for promoting intestinal development in juvenile turbot.

4 Conclusion

1. Replacing 35% of fish meal with 10% Gracilaria verrucosa, algae residue or fungi residue combined with plant proteins had no adverse effects on growth performance of juvenile turbot, while replacement with 10% Enteromorpha prolifera reduced growth performance.
2. Algae residue and fungi residue improved intestinal histological structure in juvenile turbot.

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