

## Comparison of Muscle Nutritional Composition in Juvenile *Siganus guttatus* Fed *Enteromorpha* and Artificial Feed (Postprint)

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### Abstract

This study aimed to compare the muscle nutritional composition of juvenile spotted rabbitfish (*Siganus guttatus*) fed *Enteromorpha prolifera* and artificial feed. One hundred eighty healthy juvenile spotted rabbitfish with uniform size were selected and randomly divided into two dietary groups (*Enteromorpha prolifera* group and artificial feed group), with three replicates per group (30 fish per replicate). The feeding trial lasted for 90 days. The results showed: 1) The weight gain rate, specific growth rate, and relative growth rate of juvenile spotted rabbitfish in the *Enteromorpha prolifera* group were significantly lower than those in the artificial feed group ( $P < 0.05$ ). The crude protein and crude ash contents in the *Enteromorpha prolifera* group were significantly higher than those in the artificial feed group ( $P < 0.05$ ), whereas the crude lipid content was significantly lower ( $P < 0.05$ ). No significant difference was observed in moisture content between the two groups ( $P > 0.05$ ). 2) The amino acid composition was essentially consistent between the two groups, with 17 amino acids detected in both. The essential amino acid index (EAAI) values were 76.72 and 70.05 for the *Enteromorpha prolifera* and artificial feed groups, respectively, with their composition ratios meeting the Food and Agriculture Organization/World Health Organization (FAO/WHO) standards. 3) The contents of eicosapentaenoic acid (EPA) plus docosahexaenoic acid (DHA) and omega-3 polyunsaturated fatty acids ( $\Sigma 3$ PUFA) in the *Enteromorpha prolifera* group were significantly higher than those in the artificial feed group ( $P < 0.05$ ). These results indicate that both groups of juvenile spotted rabbitfish contained various nutritional components; however, the *Enteromorpha prolifera* group exhibited superior essential amino acid and important polyunsaturated fatty acid nutrition in terms of EAAI and EPA+DHA and  $\Sigma 3$ PUFA contents. Spotted rabbitfish fed *Enteromorpha prolifera* exhibited delicious taste and possessed high edible value and health-promoting effects.

## Full Text

### Comparison of Nutritive Components in Muscles of Juvenile *Siganus guttatus* Fed with *Enteromorpha prolifera* and Artificial Feed

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**Abstract:** This experiment was conducted to compare the nutritive components in muscles of juvenile *Siganus guttatus* fed with *Enteromorpha prolifera* and artificial feed. A total of 180 healthy juvenile *Siganus guttatus* with similar body weight were divided into 2 feed groups (*Enteromorpha prolifera* group and artificial feed group), and fed with *Enteromorpha prolifera* and artificial feed, respectively. Each group contained 3 replicates and each replicate contained 30 fish. The experiment lasted for 90 days. The results showed as follows: 1) the weight gain rate, specific growth rate and relative growth rate of juvenile *Siganus guttatus* in *Enteromorpha prolifera* group were significantly lower than that in artificial feed group ( $P < 0.05$ ); the content of crude protein and crude ash of juvenile *Siganus guttatus* in *Enteromorpha prolifera* group was significantly higher than that in artificial feed group ( $P < 0.05$ ), however, the content of crude fat of juvenile *Siganus guttatus* in *Enteromorpha prolifera* group was significantly lower than that in with artificial feed group ( $P < 0.05$ ), the moisture content between two groups had no significant difference ( $P > 0.05$ ). 2) The composition of amino acids was similar between *Enteromorpha prolifera* group and artificial feed group. Seventeen kinds of amino acids were found in both two groups, the essential amino acids index (EAAI) was 76.71 and 70.05, respectively. The component of the essential amino acids (EAA) met the Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Standard. 3) The content of eicosapentaenoic acid (EPA)+ docosahexenoic acid (DHA) and  $\sum$  polyunsaturated fatty acids ( $\sum$  PUFA) of juvenile *Siganus guttatus* in *Enteromorpha prolifera* group was significantly higher than that in artificial feed group ( $P < 0.05$ ). In conclusion, the juvenile *Siganus guttatus* in two groups contains a variety of nutritional composition, however, considered the EAAI and the content of EPA+DHA and  $\sum$  PUFA, the nutrient of essential amino acids and major polyunsaturated fatty acids of juvenile *Siganus guttatus* is better in *Enteromorpha prolifera* group, *Siganus guttatus* fed with *Enteromorpha prolifera* is a nutritive and delicious food fish, and it can be an important aquaculture species with high edible value and health care function.

**Keywords:** *Siganus guttatus*; nutritive component; amino acids; fatty acids; *Enteromorpha prolifera*; artificial feed

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## Introduction

*Siganus guttatus* belongs to the order Perciformes, family Siganidae, genus *Siganus*, and is mainly distributed in tropical and subtropical Indo-Pacific waters and the South China Sea of China, ranging from coral reefs to estuarine waters [1]. As an omnivorous, euryhaline, warm-water fish, *Siganus guttatus* exhibits strong environmental adaptability, simple farming technology, low disease incidence, and fast growth rate, offering considerable economic benefits [2]. Additionally, it has the habit of feeding on attached algae on aquaculture nets, playing an important role in maintaining net cleanliness and good water quality, thus providing significant ecological benefits. These favorable economic and ecological characteristics have attracted widespread attention [3].

*Enteromorpha prolifera*, commonly known as “tai-tiao” or “qing-hai-tai,” belongs to the phylum Chlorophyta, order Ulvales, family Ulvaceae, genus *Enteromorpha* [4]. It is widely distributed in marine, estuarine, and brackish habitats at the land-sea interface [5], occurring in both southern and northern sea areas of China as a dominant species in the East China Sea [6]. In recent years, due to global warming and water eutrophication, marine green algae such as *Enteromorpha prolifera* have proliferated massively, causing frequent “green tide” events that pose corresponding hazards to surrounding aquaculture industries, particularly severe damage to sea cucumbers [7].

Currently, northern China is attempting to utilize the algae-feeding habit of *Siganus guttatus* to control excessive *Enteromorpha prolifera* growth in aquaculture waters for sea cucumbers (*Stichopus japonicus*) and other species, preventing environmental damage from overgrowth and achieving ecologically healthy farming. Systematic studies have been conducted on the growth habits, digestive enzyme activities, and antioxidant enzyme activities of *Siganus guttatus* fed with *Enteromorpha prolifera* [8-10], but the muscle nutritional composition after *Enteromorpha prolifera* consumption remains unknown. Therefore, this study employed biochemical analysis to evaluate the muscle nutritional quality of juvenile *Siganus guttatus* fed with *Enteromorpha prolifera* and artificial feed, aiming to clarify the nutritional status of *Siganus guttatus* after *Enteromorpha prolifera* consumption and further explore the application potential of *Enteromorpha prolifera* as a feed source for *Siganus guttatus*.

### 1.1 Experimental Fish

The experimental fish were juvenile *Siganus guttatus* artificially bred at the Qionghai Research Center of East China Sea Fisheries Research Institute, transported by air to Weihai, Shandong. Healthy juveniles with uniform size were selected for the experiment, with body length of  $(39.9 \pm 2.3)$  mm and body weight of  $(2.1 \pm 0.2)$  g.

### 1.2 Experimental Design and Management

The experiment was conducted in a sea cucumber farming bay at Xiaoshi Island National Marine Special Protected Area in Weihai, Shandong Province. Net cages measuring  $2.5 \text{ m} \times 1.5 \text{ m} \times 1.5 \text{ m}$  with 0.5 mm mesh size were selected for the experiment. A total of 180 juvenile *Siganus guttatus* were allocated to 2 feed groups (*Enteromorpha prolifera* group and artificial feed group), fed with fresh *Enteromorpha prolifera* and artificial feed, respectively. Each group had 3 replicates with 30 fish per replicate. The experiment ran from June 24, 2014 to September 19, 2014, lasting 90 days. Feeding occurred at 05:30, 09:00, 13:00, and 17:00 daily. Feeding behavior was observed to ensure satiation, and food residues were promptly removed. Water exchange occurred naturally with local tides. During the experiment, water temperature was 23.0–26.5 °C, salinity was 28–30, pH was  $8.0 \pm 0.5$ , and dissolved oxygen was above 5 mg/L. The conventional nutritional compositions of the two different feeds (*Enteromorpha prolifera* and artificial feed) are shown in Table 1, and the fatty acid compositions are shown in Table 2.

### 1.3 Sample Collection

At the end of the experiment, after 24 h of fasting, 10 fish were randomly sampled from each net cage, totaling 30 fish per feed group. Fish were anesthetized with 200 mg/L MS-222, and body length and weight were measured. The *Enteromorpha prolifera* group had body length of  $(6.60 \pm 0.58)$  cm and body weight of  $(9.61 \pm 0.61)$  g, while the artificial feed group had body length of  $(7.87 \pm 0.48)$  cm and body weight of  $(10.12 \pm 0.48)$  g. For analysis, 5 fish were randomly selected from the 30 fish to form one sample, totaling 6 samples. Fresh muscle samples were immediately dissected on an ice plate, minced, and mixed uniformly for determination of muscle conventional composition, amino acids, and fatty acids.

### 1.4 Sample Measurement Methods

Moisture, crude protein, crude fat, and crude ash contents were determined according to GB 5009.5–85. Amino acid contents were determined using a Biochrom-20 automatic amino acid analyzer according to GB/T 14965–1994. Fatty acid contents were determined using an Agilent-6890 gas chromatograph according to GB/T 5009.168–2003.

### 1.5 Nutritional Quality Evaluation Methods

Based on the amino acid scoring patterns recommended by the Food and Agriculture Organization (FAO)/World Health Organization (WHO) (1973) and whole egg protein amino acid patterns (both on a dry matter basis), amino acid score (AAS), chemical score (CS), and essential amino acid index (EAAI) were calculated using the following formulas:

$$\text{AAS} = (\text{aa} / \text{AA}(\text{FAO}/\text{WHO})) \times 100$$

$$\text{CS} = (\text{aa} / \text{AA}(\text{Egg})) \times 100$$

$$\text{EAAI} = \sqrt[n]{(A/AE) \times (B/BE) \times (C/CE) \times \dots \times (G/GE)} \times 100$$

Where: aa is the amino acid content in the test sample (%); AA(FAO/WHO) is the content of the same amino acid in the FAO/WHO scoring pattern (%); AA(Egg) is the content of the same amino acid in whole egg protein (%); n is the number of essential amino acids compared; A, B, C...G are the essential amino acid contents in the sample protein (%); AE, BE, CE...GE are the essential amino acid contents in whole egg protein (%).

### 1.6 Data Statistics and Processing

Before statistical analysis, all data were tested for normality using the 1-Sample K-S (Normal) procedure in SPSS 13.0 statistical software. The original data were normally distributed. Independent samples t-test was used for comparison between the two groups, and Levene's test was used for homogeneity of variance test. When variances were not homogeneous, the Mann-Whitney U test in 2 Independent Samples was used for further comparison between groups. Descriptive statistics were expressed as mean  $\pm$  standard deviation (mean $\pm$ SD), with  $P < 0.05$  considered statistically significant.

### 2.1 Growth Indices and Nutritional Composition

The growth indices and nutritional composition of juvenile *Siganus guttatus* in the *Enteromorpha prolifera* and artificial feed groups are shown in Table 3. The final body weight, weight gain rate, specific growth rate, and relative growth rate of juvenile *Siganus guttatus* in the *Enteromorpha prolifera* group were significantly lower than those in the artificial feed group ( $P < 0.05$ ). The crude protein and crude ash contents in muscle of juvenile *Siganus guttatus* in the *Enteromorpha prolifera* group were significantly higher than those in the artificial feed group ( $P < 0.05$ ), while the crude fat content was significantly lower ( $P < 0.05$ ). There was no significant difference in moisture content between the two groups ( $P > 0.05$ ).

### 2.2 Amino Acid Analysis and Quality Evaluation

The amino acid composition in muscle of juvenile *Siganus guttatus* in the two feed groups is shown in Table 4. A total of 17 common amino acids were de-

ected, including 7 essential amino acids (EAA) involved in protein synthesis: threonine (Thr), valine (Val), methionine (Met), phenylalanine (Phe), isoleucine (Ile), leucine (Leu), and lysine (Lys); 2 semi-essential amino acids (HEAA): histidine (His) and arginine (Arg); 7 non-essential amino acids (NEAA): tyrosine (Tyr), aspartic acid (Asp), glutamic acid (Glu), serine (Ser), glycine (Gly), alanine (Ala), and proline (Pro); and 1 free amino acid, taurine (Tau). The results showed that except for Gly, Met, and Leu contents, which showed no significant difference between the two groups ( $P > 0.05$ ), all other amino acid contents differed significantly ( $P < 0.05$ ). The total amino acid content (WTAA), total essential amino acid content (WEAA), total semi-essential amino acid content (WHEAA), total non-essential amino acid content (WNEAA), and total delicious amino acid content (WDAA) in the Enteromorpha prolifera group were significantly higher than those in the artificial feed group ( $P < 0.05$ ). Comparing individual amino acid contents between the two groups, all amino acids except Gly were higher in the Enteromorpha prolifera group. Among the 17 amino acids detected, Glu had the highest content, followed by Asp, Lys, and Leu, while Tau had the lowest content.

After converting the data in Table 4 to milligrams of amino acid per gram of nitrogen (by multiplying by 62.50%), the AAS, CS, and EAAI of the Enteromorpha prolifera and artificial feed groups were calculated, with results shown in Table 5. Based on the AAS and CS values, Lys was the highest in both groups, while Met+Cys was the lowest. Therefore, the first limiting amino acid in both groups was Met+Cys. The EAAI values for the Enteromorpha prolifera and artificial feed groups were 76.71 and 70.05, respectively, indicating that the essential amino acid composition was superior in the Enteromorpha prolifera group.

### 2.3 Comparison of Fatty Acid Composition

As shown in Table 6, 7 saturated fatty acids (SFA), 4 monounsaturated fatty acids (MUFA), and 11 polyunsaturated fatty acids (PUFA) were detected in muscle of juvenile *Siganus guttatus* in the Enteromorpha prolifera group, while 9 SFA, 7 MUFA, and 12 PUFA were detected in the artificial feed group. Except for C16:0, C20:0, C22:0, C18:3 $\omega$ 6, and C20:3 $\omega$ 6, which showed no significant difference between the two groups ( $P > 0.05$ ), all other fatty acids differed significantly ( $P < 0.05$ ). In terms of fatty acid composition, the  $\sum$  MUFA content was significantly higher in the Enteromorpha prolifera group ( $P < 0.05$ ), while the  $\sum$  PUFA content showed the opposite trend, being significantly higher in the Enteromorpha prolifera group ( $P < 0.05$ ). There was no significant difference in  $\sum$  SFA content between the two groups ( $P > 0.05$ ). Regarding PUFA comparison, the eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA) and  $\sum$  3 PUFA contents in the Enteromorpha prolifera group were significantly higher ( $P < 0.05$ ), while the  $\sum$  6 PUFA content showed the opposite trend, being significantly lower in the Enteromorpha prolifera group ( $P < 0.05$ ).

### 3.1 Growth and Conventional Nutritional Composition of Juvenile *Siganus guttatus* Fed with *Enteromorpha prolifera* and Artificial Feed

This experiment compared the growth and conventional nutritional composition of juvenile *Siganus guttatus* fed with *Enteromorpha prolifera* and artificial feed. The results showed that the weight gain rate, specific growth rate, relative growth rate, and crude fat content were relatively lower in the *Enteromorpha prolifera* group, but the crude protein and crude ash contents were higher. The differences in growth rate and nutritional composition between the two feed groups were closely related to the nutritional composition of the feeds and the feeding behavior of the fish. Analysis revealed that although the nutritional composition of both feeds in this study was not at the optimal level for *Siganus guttatus*, the artificial feed composition was closer to the optimal level, resulting in faster growth in the artificial feed group [9]. Additionally, the *Enteromorpha prolifera* group consumed *Enteromorpha prolifera* with low fat content, and during feeding, they continuously cut algal filaments while moving up and down in the water as the filaments broke [8], which consumed considerable energy and prevented excessive fat accumulation in the body. Consequently, the muscle fat content was relatively low in this group. Conversely, the artificial feed was more easily obtained with lower feeding energy expenditure, and the artificial feed itself had relatively high fat content, leading to greater fat accumulation and higher fat content in the artificial feed group. Fish quality is typically evaluated based on protein and fat contents. Excessively high muscle fat content not only increases dietary fat intake but also results in loose muscle texture and poor taste [14-15]. Overall, although the *Enteromorpha prolifera* group grew relatively slower, their higher protein and lower fat content conformed to the desirable “high protein, low fat” characteristic of quality fish meat.

### 3.2 Amino Acid Composition of Muscle from Juvenile *Siganus guttatus* Fed with *Enteromorpha prolifera* and Artificial Feed

Amino acids are the basic building blocks of proteins and are typically used for the synthesis of proteins, enzymes, and hormones. Some can be used directly as drugs, serve as fuel during intense exercise, or promote metabolism. Research indicates that the nutritional value and quality of protein are primarily determined by the types of amino acids and the content of essential amino acids [13]. In this study, the ratios of total essential amino acids to total amino acids (WEAA/WTAA) and total essential amino acids to total non-essential amino acids (WEAA/WNEAA) in muscle of both feed groups met the FAO/WHO ideal protein standard of approximately 40% for WEAA/WTAA and above 60% for WEAA/WNEAA [13], indicating good amino acid balance in both groups. Regarding amino acid content, glutamic acid (Glu) was the most abundant in both groups. Glu is not only an important delicious amino acid but also a crucial osmolyte that plays important roles in osmotic pressure and ion regulation in fish [16], and is an important amino acid in brain tissue metabolism involved in the synthesis of various physiologically active substances [17]. Aspartic acid



## Conclusion

Based on the experimental results, three main conclusions can be drawn. First, *Siganus guttatus* fed with *Enteromorpha prolifera* had higher muscle protein content and lower fat content, exhibiting the desirable “high protein, low fat” characteristic of quality fish meat. Second, the muscle of *Siganus guttatus* fed with *Enteromorpha prolifera* contained higher levels of essential amino acids and delicious amino acids with balanced essential amino acid composition, was rich in various important amino acid components, and demonstrated better taste, quality, and health benefits. Third, the muscle of *Siganus guttatus* fed with *Enteromorpha prolifera* was rich in various important polyunsaturated fatty acids including EPA, DPA, DHA, and ALA, indicating superior essential fatty acid nutritional quality and immune and health benefits.

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