Effects of Filamentous Algae and Eelgrass on Growth Performance and Immune Indices of Sea Cucumber Postprint

Authors: Lu Jingjing, Guo Ran, Zhao Chunlong, Cui Zhaojin, Xia Hui, Xie Wei, Wang Meixue, Yang Pinxian

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Abstract

This study aimed to investigate the effects of moss and eelgrass on the growth performance and immune indicators of sea cucumbers. A total of 720 sea cucumbers with an average weight of 0.35 g were randomly allocated into 3 groups, each consisting of 3 replicates with 80 sea cucumbers per replicate. Three different formulated feeds were prepared using moss, eelgrass, and kelp as the primary algal protein sources to feed the sea cucumbers, with the kelp group designated as the control and the moss and eelgrass groups as the experimental groups. The experimental duration was 8 weeks. The effects of moss and eelgrass on the healthy growth of sea cucumbers were evaluated by assessing growth performance, body wall nutritional composition, digestive enzyme activity, and non-specific immune indicators. The results demonstrated that the weight gain rates of the moss and eelgrass groups increased by 29.15% and 24.13%, respectively, compared to the control group, while the feed conversion ratios decreased by 10.39% and 15.58%, respectively; however, no significant differences were observed among groups ($P>0.05$). The viscera-to-body wall ratio and intestine weight ratio of sea cucumbers in the moss and eelgrass groups were significantly lower than those in the control group ($P<0.05$), whereas the body wall moisture content was significantly higher than that in the control group ($P<0.05$). The body wall crude ash content in the eelgrass group was significantly higher than that in both the moss and control groups ($P<0.05$). The intestinal amylase activity of sea cucumbers in the moss and eelgrass groups was significantly lower than that in the control group ($P<0.05$). The coelomic fluid glutathione peroxidase (GSH-PX) activity in the moss group was significantly higher than that in the control and eelgrass groups ($P<0.05$), while the activities of peroxidase (POD), superoxide dismutase (SOD), and acid phosphatase (ACP) were all elevated compared to the control and eelgrass groups, though no significant differences were detected among groups ($P>0.05$). The coelomic fluid alkaline phosphatase (ALP) activity in the eelgrass group was significantly lower than that in the control group ($P<0.05$), with no significant difference from the moss group ($P>0.05$). Therefore, under the experimental conditions of this study, the utilization of moss and eelgrass as feed ingredients in sea cucumber diets is feasible; based on growth performance indicators combined with antioxidant and non-specific immune indicators, the growth efficacy of sea cucumbers followed the order: moss group > eelgrass group > kelp group.

Full Text

Effects of Green Algae and Sargassum on Growth Performance and Immune Indices of Sea Cucumber (Apostichopus japonicus)

LU Jingjing[1], GUO Ran[1]*, ZHAO Chunlong[2], CUI Zhaojin[2], XIA Hui[1], XIE Wei[1], WANG Meixue[1], YANG Pinxin[1]

[1]Ocean College of Hebei Agricultural University, Qinhuangdao 066000, China
[2]Key Laboratory of Marine Living Resources and Environment in Hebei Province, Ocean and Fisheries Science Research Institute of Hebei Province, Qinhuangdao 066000, China

Abstract

This experiment investigated the effects of dietary green algae and Sargassum on growth performance and immune indices of sea cucumber (*Apostichopus japonicus*). A total of 720 juvenile sea cucumbers with an average body weight of 0.35 g were randomly allocated into three groups with three replicates each (80 individuals per replicate). Three experimental diets were formulated using green algae, Sargassum, or kelp as the primary algal protein source, with the kelp diet serving as the control. The green algae and Sargassum groups constituted the experimental treatments. After an 8-week feeding trial, growth performance, body wall nutrient composition, digestive enzyme activities, and non-specific immune indices were evaluated to assess the impact on healthy sea cucumber development. The results demonstrated that compared with the control group, the weight gain rates in the green algae and Sargassum groups increased by 29.15% and 24.13%, respectively, while feed conversion ratios decreased by 10.39% and 15.58%, though these differences were not statistically significant ($P>0.05$). Both experimental groups exhibited significantly lower viscera-to-body-wall ratios and intestine-weight-to-body-weight ratios ($P<0.05$), along with significantly higher body wall moisture content ($P<0.05$). The Sargassum group showed significantly elevated crude ash content in the body wall compared to both the control and green algae groups ($P<0.05$). Intestinal amylase activity was significantly reduced in both the green algae and Sargassum groups relative to the control ($P<0.05$). Notably, coelomic fluid glutathione peroxidase (GSH-Px) activity in the green algae group was significantly higher than in the control and Sargassum groups (P<0.05), while peroxidase (POD), superoxide dismutase (SOD), and acid phosphatase (ACP) activities were also elevated but without significant differences (P>0.05). The Sargassum group displayed significantly lower alkaline phosphatase (ALP) activity in coelomic fluid compared to the control (P<0.05), though it did not differ significantly from the green algae group (P>0.05). These findings indicate that under the experimental conditions, both green algae and Sargassum are viable dietary ingredients for sea cucumber feed formulations. Based on comprehensive evaluation of growth performance, antioxidant capacity, and non-specific immune indices, the overall growth efficacy ranked as: green algae group > Sargassum group > kelp group.

Keywords: sea cucumber; green algae; Sargassum; growth performance; non-specific immunity

Introduction

Apostichopus japonicus, belonging to the phylum Echinodermata and class Holothuroidea, represents one of China' s primary edible sea cucumber species, renowned for its high nutritional and medicinal value and historically acclaimed as the foremost among the "Eight Treasures of the Sea" [1]. In recent years, rising living standards and expanding market demand have substantially increased sea cucumber consumption, thereby stimulating rapid development of the sea cucumber aquaculture industry [2]. This expansion has intensified research focus on sea cucumber feed formulations. Feed quality directly determines the success of sea cucumber cultivation operations. However, the current market faces challenges including increasing feed demand, inconsistent quality among commercial formulated feeds, shortages of premium raw materials, and a lack of well-formulated, nutritionally complete products, all of which represent potential constraints on industry development [3].

In sea cucumber aquaculture, large macroalgae such as kelp (*Laminaria* spp.), *Sargassum thunbergii*, and other seaweeds serve as primary dietary ingredients, but demand has surged annually. Overexploitation and water pollution have created supply shortages of these valuable algal resources, necessitating identification of suitable alternative ingredients. Green algae, also known as "qingnitai," collectively refers to dozens of filamentous green algae including *Spirogyra*, *Cladophora*, *Zygnema*, and *Hydrodictyon* [4]. While excessive green algae in ponds can degrade water quality and hinder aquatic animal growth—prompting removal through manual, chemical, or biological methods [5-6]—certain beneficial species exist. Marine green algae primarily comprise soft red algae, *Enteromorpha*, and *Ulva*, containing 7-8% crude protein in dry matter with relatively stable nutritional profiles. Previous studies have demonstrated positive effects when incorporating marine green algae into pig and fish feeds, though its potential as an algal protein source in sea cucumber formulations remains unexplored. Meanwhile, large macroalgae have been successfully applied as feed ingredients in aquaculture with favorable outcomes [7]. *Sargassum* (commonly called "dayezao" in Chinese) exhibits exceptional salt tolerance and contains phosphatidylcholine and phosphatidylethanolamine components [8]. Research on *Sargassum* extract has revealed antibacterial activity against pathogens causing skin ulcer syndrome in sea cucumbers [9], and Tang et al. [10] found that mixed *Enteromorpha* and *Sargassum* powder could effectively replace commercial algal meals in sea cucumber feed. This study aimed to design cost-effective feed formulations by replacing expensive conventional ingredients with inexpensive green algae and *Sargassum*, thereby promoting sea cucumber growth while reducing feed costs.

Materials and Methods

Experimental Diets

Kelp was purchased from Qinhuangdao Tianqiao Seafood Market, green algae were collected from sea cucumber culture ponds at a tiger puffer farm (dried and pulverized), and *Sargassum* was obtained from Xinkaikou Fishery Supply Store in Changli County. The primary ingredients, including kelp, green algae, and *Sargassum*, were ground using a pulverizer and passed through a 60-mesh sieve before diet preparation. The experimental diet formulations and nutritional levels are presented in Table 1 . Ingredients were mixed using a stepwise addition method and thoroughly blended in a mixer (HYJ-30, Beijing Huanya Tianyuan Machinery Technology Co., Ltd.), then stored at -20 °C until use.

Experimental Design and Husbandry

Juvenile sea cucumbers were procured from Shandong Anyuan Aquatic Products Co., Ltd. and acclimated in glass aquaria for two weeks prior to the experiment. A total of 720 healthy individuals with uniform size, similar coloration, and average body weight of 0.35 g were randomly selected and distributed into three dietary groups with three replicates each (80 individuals per replicate). The experiment was conducted in nine glass aquaria (0.4 m $\times$ 0.5 m $\times$ 0.6 m). Throughout the 8-week trial, sea cucumbers were reared under dark conditions and fed daily at 10:00 at 3-5% of body weight. Natural seawater from the Bohai Bay (Qinhuangdao Port area) was used after sedimentation and filtration. Water temperature was maintained at 17 $\pm$ 1 °C, salinity at 30.5-31.5, dissolved oxygen above 7 mg/L, and pH at 8.0 $\pm$ 0.2. The tank bottom was siphoned daily with water exchange of 2/3 to 3/4 of the total volume. Feeding behavior and mortality were monitored and recorded throughout the experiment.

Sample Collection

At the conclusion of the trial, all sea cucumbers were fasted for 24 hours before final counting and weighing. From each replicate, 11 individuals were selected for individual weight and length measurement. Coelomic fluid was extracted from the abdominal one-third region using a 2.5 mL syringe. The complete intestinal tract was dissected out, and intestinal length was measured. Intestinal and body wall weights were recorded to calculate intestinal indices and viscera-to-body-wall ratios. Coelomic fluid was centrifuged at 2,500 × g, and the supernatant was stored at 4 °C for immune index analysis. Intestinal and body wall samples were rapidly frozen in liquid nitrogen and stored at -80 °C for subsequent analysis of digestive enzyme activities and nutrient composition.

Index Determination

Growth performance indices were calculated using the following formulas:

- Weight gain rate (WGR, %) = (Wt - Wo) / Wo × 100
- Specific growth rate (SGR, %/d) = (ln Wt - ln Wo) / t × 100
- Protein efficiency ratio (PER, %) = (Wt - Wo) / (F × P) × 100
- Feed conversion ratio (FCR) = F / (Wt - Wo)
- Viscera-to-body-wall ratio (%) = Wv / Wb × 100
- Intestine-weight-to-body-weight ratio = Wi / Wt
- Intestine-length-to-body-length ratio = Li / L

Where Wo represents initial body weight (g), Wt represents final body weight (g), Wv represents viscera weight (g), Wi represents intestine weight (g), Wb represents body wall weight (g), Li represents intestine length (cm), L represents body length (cm), F represents feed intake (g), P represents dietary protein content (%), and t represents experimental duration in days.

Body wall nutrient composition was analyzed using standard methods: moisture content by drying at 105 °C to constant weight, crude ash by muffle furnace incineration at 550 °C, crude protein by Kjeldahl nitrogen determination, and crude lipid by Soxhlet extraction.

Digestive and immune indices were measured using commercial assay kits (Nanjing Jiancheng Bioengineering Institute) according to the following methods: amylase (iodine-starch colorimetry), lipase (turbidimetry), pepsin (Folin reagent colorimetry), peroxidase (phenol colorimetry), glutathione peroxidase (dithiobisnitrobenzoic acid method), total superoxide dismutase (xanthine oxidase method), and phosphatase activity (potassium ferricyanide method).

Statistical Analysis

All data are expressed as mean ± standard deviation (SD). Statistical analysis was performed using SPSS 17.0 software with one-way ANOVA. Significance level was set at $P < 0.05$.

Results

Effects of Different Diets on Growth Performance

As shown in Table 2 , the weight gain rates of sea cucumbers in the green algae and Sargassum groups increased by 29.15% and 24.13% compared to the control group, respectively, though these differences were not statistically significant ($P > 0.05$). Specific growth rate, feed intake, and protein efficiency ratio were also higher in both experimental groups than in the control, but without significant differences ($P > 0.05$). Feed conversion ratios in the green algae and Sargassum groups decreased by 10.39% and 15.58% relative to the control, respectively, but again showed no significant differences ($P > 0.05$). Notably, the viscera-to-body-wall ratios in both experimental groups were significantly lower than that of the control group ($P < 0.05$).

Effects of Different Diets on Body Wall Nutrient Composition

Table 3 presents the body wall nutrient composition data. Moisture content in the body wall was significantly higher in both the green algae and Sargassum groups compared to the control ($P < 0.05$). Crude ash content in the Sargassum group was significantly elevated above that of both the control and green algae groups ($P < 0.05$), while the green algae group did not differ significantly from the control in this parameter ($P > 0.05$). No significant differences were observed among groups in body wall crude protein or crude lipid content ($P > 0.05$).

Effects of Different Diets on Intestinal Indices

The intestinal indices are summarized in Table 4 . Both the green algae and Sargassum groups exhibited significantly lower intestine-weight-to-body-weight ratios compared to the control ($P < 0.05$), though no significant difference existed between the two experimental groups ($P > 0.05$). Intestine-length-to-body-length ratios did not differ significantly among any groups ($P > 0.05$).

Effects of Different Diets on Intestinal Digestive Enzyme Activities

According to Table 5 , no significant differences were detected among groups in intestinal lipase or protease activities ($P > 0.05$). However, intestinal amylase activity was significantly lower in the green algae group compared to the control ($P < 0.05$), and the Sargassum group showed even greater reduction, with activity significantly lower than both the control and green algae groups ($P < 0.05$).

Discussion

Effects on Growth Performance

Nutritional composition, palatability, and feed attractiveness are critical factors influencing animal growth [11-12]. Zhu et al. [13] established that the optimal dietary protein requirement for sea cucumber ranges from 18.21% to 24.18%, with an optimal lipid requirement of 5%. In this study, experimental diets were formulated using green algae and Sargassum as primary ingredients to achieve protein and lipid levels of approximately 22% and 5%, respectively, with kelp as the control. The results demonstrated that both experimental groups achieved higher weight gain rates and protein efficiency ratios alongside lower feed conversion ratios compared to the control, confirming the feasibility of these formulations. Furthermore, the significantly lower viscera-to-body-wall ratios in the green algae and Sargassum groups indicate that these ingredients promote more efficient body wall growth, suggesting superior nutrient partitioning toward tissue deposition.

Effects on Body Wall Nutrient Composition

Natural sea cucumbers primarily consume sediment, exhibiting distinct energy allocation patterns characterized by high fecal energy loss and relatively low growth energy allocation [14-15]. Although feed-derived energy is substantial, a considerable portion is expended as fecal energy and for other metabolic activities, while the remainder supports various growth demands [16]. Dietary fiber at appropriate levels can enhance gastrointestinal motility and facilitate nutrient absorption. In this study, the green algae group showed higher feed intake than both the control and Sargassum groups, with slightly elevated body wall crude protein and marginally reduced crude lipid content. This may be attributed to the suitable fiber content and favorable palatability of green algae and Sargassum, which could enhance protein digestibility and deposition efficiency. The significantly higher crude ash content in the Sargassum group suggests a potential role in promoting mineral deposition, though this warrants further investigation.

Effects on Intestinal Indices and Digestive Enzyme Activities

The intestine-weight-to-body-weight ratio serves as an indicator of feeding activity, typically being higher in actively feeding individuals than in starved ones, and decreasing as development progresses [17]. The significantly lower intestine-weight-to-body-weight ratios in the green algae and Sargassum groups, combined with superior weight gain and specific growth rates, indicate that these diets support more advanced developmental status and growth efficiency compared to the kelp-based control. The intestine represents the primary digestive organ in sea cucumbers, secreting various digestive enzymes to facilitate nutrient absorption [18]. Gangadhara et al. [19] demonstrated that dietary composition changes can alter digestive enzyme activities, while Guo [2] suggested that sea cucumbers can adapt their intestinal physiology and enzyme activities to different feed sources. In this study, protease and lipase activities did not differ significantly among groups, but amylase activity was significantly reduced in both experimental groups, consistent with findings from Li et al. [11] using different feed ingredients.

Effects on Non-Specific Immune Indices

As higher invertebrates, sea cucumbers lack a specific immune response system and rely primarily on non-specific defense mechanisms, including phagocytosis by coelomocytes, respiratory burst activity, and various immune factors [20-21]. Glutathione peroxidase, peroxidase, and superoxide dismutase constitute important antioxidant enzymes that decompose peroxides, eliminate hydrogen peroxide and reactive oxygen species, and scavenge free radicals [22]. This study revealed no significant differences in coelomic fluid peroxidase or total superoxide dismutase activities among groups. However, the green algae group exhibited significantly elevated glutathione peroxidase activity, suggesting enhanced capacity to detoxify harmful peroxides into non-toxic hydroxyl compounds and improved immune function. Alkaline phosphatase (ALP) and acid phosphatase (ACP) are lysosomal marker enzymes and crucial immune factors that not only promote calcium-phosphorus metabolism but also indirectly reflect tissue damage and serve as primary indicators for evaluating non-specific immunity [23]. While the physiological and pathological reasons for the significantly reduced ALP activity in the Sargassum group remain unclear, no significant differences were observed in ACP activity among treatments.

In conclusion, under the experimental conditions, both green algae and Sargassum demonstrate feasibility as dietary ingredients for sea cucumber feed. Based on comprehensive evaluation of growth performance, antioxidant capacity, and non-specific immune indices, the overall efficacy ranked as: green algae group > Sargassum group > kelp group.

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Note: Figure translations are in progress. See original paper for figures.