Aquaculture 470 (2017) 157–163

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Growth, feed utilization and energy budgets of the sea cucumber *Apostichopus japonicus* with different diets containing the green tide macroalgae *Chaetomorpha linum* and the seagrass *Zostera marina*

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ARTICLE INFO

Article history:
Received 24 June 2016
Received in revised form 27 November 2016
Accepted 27 December 2016
Available online 29 December 2016

Keywords:
Bloom-forming macroalgae
Chaetomorpha linum
Sea cucumber
Apostichopus japonicus
Eelgrass
Food source
Growth

ABSTRACT

Apostichopus japonicus is one of the main aquaculture species in northern China. The green tide macroalgae *Chaetomorpha linum* and the seagrass *Zostera marina* occur in this region. Our previous study has suggested that *Z. marina* detritus can be a food source for *A. japonicus*. In the present study, we carried out a laboratory simulation experiment for 60 days to understand whether *C. linum* can also be utilized to fulfill the nutrient demands of *A. japonicus*. The powdered *C. linum* and *Z. marina* detritus, mixed in five ratios (100:0; 75:25; 50:50; 25:75; 0:100), were fed to sea cucumbers along with muddy sediment in a fixed proportion. *Sargassum thunbergii* (40%) combined with the muddy sediment (60%) was also used as a standard reference/control diet. Results showed that the specific growth rates (SGRs), food utilizing efficiencies and energy budgets of *A. japonicus* were strongly influenced by the ratio of *C. linum* and *Z. marina* in their diets. The growth energy deposited in the bodies of sea cucumbers fed higher proportions of *C. linum* was significantly higher than those fed higher proportions of *Z. marina*. Furthermore, sea cucumbers fed on *C. linum* and *S. thunbergii* had similar growth performances. This study indicated that *C. linum* could replace *S. thunbergii* as a food source for *A. japonicus*.

Statement of relevance: Because of its high nutritive and medical properties, the sea cucumber *Apostichopus japonicus* has become a highly valuable fishery and aquaculture species in Asia. Influenced by eutrophication in coastal waters, blooming events of the green tide algae *Chaetomorpha linum* are increasing around the world, including some natural habitats for *A. japonicus* in northern China. However, whether *C. linum* can also be utilized to fulfill the nutrient demands of sea cucumbers, such as *A. japonicus*, is unknown. We carried out a laboratory simulation experiment for 60 days to study this question. Our results showed that sea cucumbers fed on *C. linum* and *Sargassum thunbergii* had similar performances. Due to its higher protein and lower cellulose contents, *C. linum* was easier for *A. japonicus* to digest and absorb, and more energy per unit of food was converted to growth. This study indicated that *C. linum* could act as a food source for *A. japonicus*. The results of this research are also beneficial for promoting the collection and control of *C. linum*.

1. Introduction

The sea cucumber *Apostichopus (Stichopus) japonicus* Selenka, belonging to Echinodermata, Holothuroidea, Aspidochirotida, is an epibenthic, temperate species and is mainly distributed in the shallow seas of the North Pacific Ocean (Sloan, 1985; Liao, 1997). Because of its high nutritive and medical properties, the sea cucumber *A. japonicus* has become a highly valuable fishery and aquaculture species in Asia (Sun et al., 2004; Okorie et al., 2008; Seo and Lee, 2011; Yang et al., 2015). *A. japonicus* is a deposit feeder, obtaining nutrition from sedimentary organic detritus, including benthic microbes and organic fragments from marine plants and animals (Choe, 1963; Yingst, 1976; Moriarty, 1982; Zhang et al., 1995; Kang et al., 2003; Zhou et al., 2006; Yuan et al., 2006; Yu et al., 2014). Diets containing distinct seaweed organic materials can result in markedly different growth, digestion and energy allocation of sea cucumbers (Yuan et al., 2006; Xia et al., 2012; Bai et al., 2016). The brown algae *Sargassum* spp. is the most favorite fodder for the culture of sea cucumber (Asha and Muthiah, 2007). However, the large-scale collection results in the inadequate supply of *Sargassum* spp. Therefore, it is necessary to find substitute macroalgae for sea cucumber fodder. Gao et al. (2011) found that the red algae

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http://dx.doi.org/10.1016/j.aquaculture.2016.12.035
0044-8486/

Gracilaria lemaneiformis is more preferable to nutritional requirements of sea cucumbers relative to the traditional sea cucumber feed *S. thunbergii*. The bloom-forming species *Enteromorpha prolifera* that has leaded to the largest green tide in China has also been found to be an alternative food source for juvenile sea cucumbers (Guo et al., 2011).

In temperate China, Zosteraceae seagrass is widely distributed (Yang and Wu, 1981; Zhou et al., 2015; Zhang et al., 2015; Lin et al., 2016); and *Zostera marina* meadows are one of the most important coastal habitats for various marine organisms, including the sea cucumber *A. japonicus* (Liu et al., 2013; Zhou et al., 2015). Many deposit-feeders live on detritus stemming from seagrass decomposition. Researchers have found large amounts of seagrass detritus in the stomach contents of *A. japonicus* inhabiting seagrass beds, which implies the detritus is a food source for this species (Hauksson, 1979; Sui, 1988).

In recent years, with coastal water eutrophication aggravated by anthropogenic sewage discharge, the explosive growth of *Chaetomorpha linum* has led to many "green tide blooms"(Krause-Jensen et al., 1999; McGlathery et al., 2007; Zhang et al., 2014; Cooke et al., 2015), and have caused serious environmental problems and threatened the health of seagrass ecosystems in northern China (Han and Liu, 2014; Zhang et al., 2014). Thick algal mats of *C. linum* covering the seafloor may have profound influences on local benthic biogeochemical processes (e.g. carbon mineralization, oxygen consumption, denitrification and nutrient dynamics) and community structure of ecosystems (Krause-Jensen et al., 1999; Hansen and Kristensen, 1998; Menendez et al., 2002; Green et al., 2014).

Swan Lake is a coastal lagoon located in Weihai, northern China, in which the eelgrass *Z. marina* can be found in large abundances (Zhou et al., 2015). The dense seagrass meadow of this lagoon provides suitable habitat for abundant sea cucumbers, bivalves, octopuses, fish and crabs. In the past few years, considerable growth of *C. linum* mats has been observed each summer, replacing seagrass in several areas of the lagoon (Zhang et al., 2014). It has been reported that eelgrass detritus can be a food source for sea cucumbers (Liu et al., 2013), and detritus of *Z. marina* and *C. linum* have been found in the alimentary canals of sea cucumbers in Swan Lake. However, whether *C. linum* can be utilized to fulfill the nutrient demands of sea cucumbers is unknown. In order to clarify this, we mixed the powdered *C. linum* and *Z. marina* detritus with muddy sediment, which is an essential food source of sea cucumbers in their natural environment (Slater and Jeffs, 2010), to form experimental diets in the present study. To provide a standard reference diet, we mixed muddy sediment with *Sargassum thunbergii*, which is a main ingredient of the formulated diet applied in sea cucumber cultivation (Yuan et al., 2006; Slater et al., 2009). We compared the growth, feed utilization and energy budgets of *A. japonicus* fed with these different diets to ascertain whether *C. linum* provided a better food source.

2. Materials and methods

2.1. Diet preparation and design

Six mixed diet treatments were used in this experiment. Five diets contained muddy sediment mixed with the powdered *Chaetomorpha linum* and *Zostera marina* detritus in different proportions, and the sixth was a reference diet containing a mixture of muddy sediment and *Sargassum thunbergii* (Table 1).

Fresh *C. linum* used in this experiment was collected from Swan Lake (37° 21′ N, 122° 34′ E), Weihai (Fig. 1). *Z. marina* detritus was composed of fallen autumn leaves collected from the shore of Swan Lake. Muddy sediment was collected from the surface of non-vegetated seafloor in the coastal area of Laizhou Bay, northern China. Diet components were dried, ground and sieved through a 0.08 mm mesh, and the powders obtained were mixed well according to treatment proportions. The nutrition contents of the four diet ingredients, i.e. *C. linum*, *Z. marina*, *S. thunbergii* and muddy sediment, were presented in Table 2. The nutritional composition, including percentages of dry matter, protein, lipid and ash, as well as total energy of each diet was calculated (Table 3).

Table 1
Six experimental diets and their formulas.

Diets	Ingredient proportion			
	C. linum (%)	*Z. marina* (%)	*S. thunbergii* (%)	Muddy sediment (%)
Diet 1	40	0	0	60
Diet 2	30	10	0	60
Diet 3	20	20	0	60
Diet 4	10	30	0	60
Diet 5	0	40	0	60
Diet 6	0	0	40	60

2.2. Experimental implementation

The experiment was carried out in the laboratory at the Institute of Oceanology, Chinese Academy of Sciences, Qingdao, P. R. China, from December 5th, 2015 to February 4th, 2016. Sea cucumbers were collected from Swan Lake, Weihai, on November 19th, 2015 when the water temperature was 13.5 °C. Prior to the initiation of the experiment, sea cucumbers were acclimated for two weeks in the experimental culture system, which consisted of plastic containers measuring 50 × 40 × 30 cm. The *S. thunbergii* diet (Diet 6) was provided as feed during acclimation.

After 2 d of starvation, 72 acclimated sea cucumbers with initial wet body weights of 10.45 ± 0.35 g ind^{-1} were randomly chosen and distributed evenly into 24 plastic containers (four replicates for each of the six experimental groups). During the experiment, aeration was provided continuously and one-third the volume of the water in each container was changed daily to ensure good water quality. The culture temperature was 16 ± 0.5 °C, which is within the optimum range for this species (Chen, 2004). Other water conditions were pH 7.8–8.2; salinity 30–32 ppt and dissolved oxygen maintained above 5.0 mg L^{-1}. Dietary intake was monitored to ensure experimental animals always had an excess of food available.

2.3. Sample collection and measurement

The wet and dry weights of a further 10 sea cucumbers of the same size as the aforementioned experimental animals were measured to estimate water content of experimental animals. Sea cucumber feces and residual food was removed and collected by siphon once per day (at 08:00) and dried at 60 °C to constant weight for further disposal and analysis. At the end of experiment, the sea cucumbers were processed as outlined previously after a final starvation period of 2 d. Residual food, sea cucumbers and their feces from the same container were gathered as one sample.

The ash content of dried food and feces was estimated by combusting (500 °C for 3 h) dried and pre-weighed samples. The nitrogen content of diets, feces, and sea cucumber samples was determined using an elemental analyzer (EA-IRMS, Thermo Finnigan MAT Delta-plus). The energy content of the diets, feces and sea cucumber samples was measured using a PARR1281 Calorimeter (PARR Instrument Company, Moline, IL, USA). Proximate composition of diets and nutrition contents of ingredients were determined according to the technical specifications of AOAC (1990). All measurements were obtained in duplicate to ensure accuracy.

2.4. Data calculation and statistical analysis

The specific growth rate (SGR), ingestion rate (IR), feces production rate (FPR), food conversion efficiency (FCE) and apparent digestive ratio (ADR) of sea cucumbers in each plastic container were calculated