Aquaculture 466 (2017) 26–32

Contents lists available at ScienceDirect

Aquaculture

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

# Effects of feeding frequency and density on growth, energy budget and physiological performance of sea cucumber *Apostichopus japonicus* (Selenka)

Bin Xia [a,b], Yichao Ren [b], Jiying Wang [a,*], Yongzhi Sun [a], Zhendong Zhang [c]

[a] Key Laboratory of Marine Ecological Restoration, Shandong Marine Resource and Environment Research Institute, Yantai, Shandong 264006, China
[b] Marine Science and Engineering College, Qingdao Agricultural University, Qingdao, Shandong 266109, China
[c] National Fishery Technique Extension Center, Beijing 100125, China

A R T I C L E   I N F O

*Article history:*
Received 14 June 2016
Received in revised form 14 September 2016
Accepted 23 September 2016
Available online 26 September 2016

*Keywords:*
*Apostichopus japonicus*
Feeding strategy
Growth performance
Endocrine response
Energy budget

A B S T R A C T

Feeding frequency and density are two of the most important factors that directly affect the growth and physiology of sea cucumber *Apostichopus japonicus*. In the present study, a 60-day experiment was conducted to evaluate the effects of feeding frequency (1 time, 2 times and 3 times per day) and stocking density (D10, D20, D40 and D60) on growth performance, feed utilization, endocrine response and energy budget of sea cucumber. The results revealed that there was no significant interaction between feeding frequency and stocking density on final weight and specific growth rate (SGR). The maximum SGR occurred at treatment of stocking density D10 with feeding thrice per day. Stepwise multiple regression analysis showed that $SGR_W$ and $SGR_E$ increased with increasing feeding frequency, and decreased with the increase of stocking density. Density had significant influence on coefficient of variation (CV) for the sea cucumber body weight. For D10 and D20, no significant difference in CV was found between different feeding frequencies. Feed intake, energy intake and feces production rate were significantly affected by feeding frequency, stocking density and their interaction. Density also had significant influence on food conversion efficiency and protein efficiency ratio. Apparent digestibility coefficients of crude protein and crude lipid exhibited remarkable descending trends as stocking density increased. The energy for growth decreased and energy required for metabolism increased with the increase of stocking density, suggesting that crowding stress modified their energy allocation by inhibiting the accumulation of growth energy and accelerating energy consumption of sea cucumber. Cortisol level in coelomic fluid of sea cucumber significantly increased with the increase of stocking density, which could generate energy to satisfy the increasing demand of stress-related energy. Lactate level significantly elevated, and glucose level in coelomic fluid and glycogen in muscle showed remarkable descending trends from D10 to D60, implying long-term energy consumption under high stocking density. Feeding frequency had significant influence on glucose, glycogen and cortisol levels. To some extent, increasing feeding frequency could effectively reduce coefficient of variation of sea cucumber, especially under high stocking density.

*Statement of relevance:*

- In the present study, a 60-day experiment was conducted to evaluate the effects of feeding frequency (1 time, 2 times and 3 times per day) and stocking density (D10, D20, D40 and D60) on growth performance, feed utilization, endocrine response and energy budget of sea cucumber.
- Increasing feeding frequency could effectively reduce coefficient of variation of sea cucumber.
- The present study provided valuable information for the intensive culture of *A. japonicus*.

## 1. Introduction

Sea cucumber *Apostichopus japonicus* (Selenka) (Echinodermata: Holothuroidea) has been used as a traditional remedy for wound healing, and extensively believed to be aphrodisiac and curative effects

---

[*] Corresponding author.
*E-mail address:* ytwjy@126.com (J. Wang).

http://dx.doi.org/10.1016/j.aquaculture.2016.09.039
0044-8486/

(Wu et al., 2015a). The natural distribution of *A. japonicus* covers the subtidal zone from 35°N to 44°N along the coast of Russia, China, Japan and Korea (Sloan, 1984; Yuan et al., 2009). With the overfishing of natural resources and increasing market demand, the farming scale of sea cucumber has been rapidly expanded in the last decades (Sun et al., 2012b). The total production of *A. japonicus* has reached 201,000 t in 2014 with an increase of 96.7% compared to that in 2009 (MOAC, 2010 and 2015).

As an obligate deposit-feeding species (Liao, 1980), sea cucumber might take up organic matter in sediment as food sources, e.g. bacteria, prozotoa, benthic microalgae, detritus of macroalgae and sea grass (MacTavish et al., 2012; Slater and Jeffs, 2010). Previous studies regarding various aspects of *A. japonicus*, include its genetics (Du et al., 2012; Li et al., 2009), energetic (Bai et al., 2015; Yuan et al., 2009), nutrition (Xia et al., 2015a; Xia et al., 2013) and larval development (Li et al., 2010; Sun et al., 2012a), etc. However, few studies have been conducted on feeding strategy of sea cucumber (An et al. 2007), and it is crucial to set feeding ration and frequency with respect to feeding habit under laboratory condition and in aquaculture practice.

An organism's morphology, behaviour, feeding and physiological characteristics tend to be adapted for maximum net energetic benefit, which is interpreted as the optimal foraging theory (Werner and Mittelbach, 1981; Wu et al., 2015b). Previous studies showed that increasing feeding frequency could improve growth, feed utilization and physiological performance of aquatic animals, e.g. fish (Dwyer et al., 2002; Wang et al., 2007) and invertebrates (Cárcamo, 2015; Cárvalho et al., 2006). Density is another important factor affecting the growth and endocrine response of aquatic animals (Façanha et al., 2016; Pei et al., 2012). High density can stimulate food competition, social hierarchy, depensatory growth, size heterogeneity and cannibalism (Ribeiro et al., 2015; Raimondo et al., 2013). In the present study, we hypothesised that increasing feeding frequency could effectively improve growth performance, reduced variation in individual growth and optimize energy allocation of sea cucumber, especially under high stocking density. The objective of this study was to investigate the effects of feeding frequency and stocking density on growth, energy budget and physiological performance of sea cucumber, providing valuable information for the intensive culture of *A. japonicus*.

## 2. Materials and methods

### 2.1. Experimental design

Juvenile sea cucumbers (age 2) of similar wet weight of 22.14 ± 2.01 g were collected from a local farm in Dongying City, Shandong Province, and transported to the laboratory immediately. All animals were acclimated in a large tank for 3 weeks at 20 ± 0.5 °C, salinity was 30–32 PSU, dissolved oxygen was above 6.5 ml $l^{-1}$ and a 14 h light: 10 h dark photoperiod, which were same with the experimental conditions. In this study, 05:00–19:00 was defined as “daytime” and 19:00–05:00 was defined as “nighttime”. During the period of the acclimation, the stocking density was approximately 500 g $m^{-2}$, and the sea cucumbers were fed once (at 16:00) per day.

After acclimation, the sea cucumbers were randomly divided into 3 groups, which were fed once (at 16:00), twice (at 08:00 and 16:00) and thrice (at 08:00, 16:00 and 24:00) per day, respectively. The feeding time was set by daily activity rhythm (Dong et al., 2011) and feeding habit (Sun et al., 2015) of sea cucumber. Each group contained four treatments of stocking densities, i.e., 10, 20, 40 and 60 individuals per cylinder aquarium (~400 l capacity, 100 cm height × 70 cm diameter), represented as D10, D20, D40 and D60, respectively. The corresponding densities were also expressed as 553.5, 1120.2, 2232.6 and 3335.7 g $m^{-2}$. Each treatment had three replicates. The experiment lasted for 60 days. During the acclimation and experimental period, the sea cucumbers were fed with dry pellets (22.90% protein, 2.31% lipid, 38.21% ash and 12.06 kJ $g^{-1}$ energy) and up to 5% of their total biomass per day. According to our previous studies (Liu et al., 2009; Xia et al., 2015b), this ration size could ensure the satiation of this species and minimize the feed waste. Each meal amount was equal for sea cucumber those were fed more than once each day. Feeding ration was adjusted once every ten days based on its growth performance. Uneaten feed residues and feces were collected by siphoning before next feeding, and dried at 60 °C to a constant weight for further analysis (Yuan et al., 2006). The weight of uneaten feed was adjusted by the leaching ration of diets in water (Shi et al., 2015). All diets contained 500 mg $kg^{-1}$ yttrium oxide as an inert marker for determining digestibility.

### 2.2. Sample collection and determination

At the end of the experiment, the sea cucumbers were starved for 24 h prior to sampling. Ten sea cucumbers were collected randomly from each aquarium, weighted and counted for calculation of growth performance. After weighting, the sea cucumbers were dried at 60 °C individually for at least 72 h to a constant weight to determine feed utilization. The dried samples were ground to fine and homogeneous powder, which was tightly sealed in a glass Petri dish and stored at −80 °C for further analysis.

Proximate composition of the diets was analyzed according to the standard methods of AOAC (1995). Crude protein ($N \times 6.25$) was determined by the Kjeldahl method after acid digestion. Crude lipid was determined by the ether-extraction method. Moisture was determined by oven drying at 105 °C for 24 h. Ash was determined by using a muffle furnace at 550 °C for 6 h. Apparent digestibility coefficients (ADC) were measured following the method by Xia et al. (2015a). Yttrium concentrations in the diets and feces were determined with an inductively coupled plasma atomic emission spectrophotometer (ICP-OES, VIATA-MPX) after perchloric acid digestion. The energy content of the diets, feces and animal samples were measured by a bomb calorimeter (Parr 6100, Parr Instrument Company).

Coelomic fluid was sampled by puncturing the abdomen with a 1 ml disposable syringe and then separated the supernatant by centrifugation (200 rpm) for 10 min at 4 °C and stored at −80 °C for further analysis. Glucose in coelomic fluid was determined using Glucose Diagnostic Kits (Rsbio, China). Cortisol level in coelomic fluid was determined using Lodine [$^{125}$I]-Cor RIA Kits (Jiuding Diagnostic, China) by radioimmunoassay and lactate was determined enzymatically using Sigma Diagnostic Kits (Sigma, USA). Glycogen in muscle was determined according to Chen (2013) with assay kits (Nanjing Jiancheng Biotech Company, China).

### 2.3. Data calculation

Weight gain, specific growth rate in terms of weight ($SGR_W$) and energy ($SGR_E$), coefficient of variation for body weight (CV), feed intake (FI), feces production rate (FPR), food conversion efficiency (FCE) and protein efficiency ratio (PER) were calculated as follow:

$$\text{Weight gain }(\%) = (W_f - W_i)/W_f \times 100$$

$$SGR_W\left(\%\ d^{-1}\right) = \ln(W_f/W_i)/t \times 100$$

$$SGR_E\left(\%\ d^{-1}\right) = \ln(E_f/E_i)/t \times 100$$

$$CV\ (\%) = SD/W_m \times 100$$

$$FI\ \left(g\ g^{-1}\ d^{-1}\right) = I/[(W_f + W_i)/2 \times t]$$

$$FPR\ \left(g\ g^{-1}\ d^{-1}\right) = F/[(W_f + W_i)/2 \times t]$$

$$FCE\ (\%) = (W_f - W_i)/I \times 100$$