Journal of Thermal Biology 60 (2016) 155–161

Contents lists available at ScienceDirect

Journal of Thermal Biology

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

Impact of water temperature on the growth and fatty acid profiles of juvenile sea cucumber *Apostichopus japonicus* (Selenka)

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ARTICLE INFO

Article history:
Received 25 March 2016
Received in revised form
5 July 2016
Accepted 6 July 2016
Available online 7 July 2016

Keywords:
Echinoderms
Thermal requirements
Growth
Lipids
HUFA

ABSTRACT

The present study determined the changes in the fatty acid (FA) profiles of juvenile sea cucumber *Apostichopus japonicus* in response to the varied water temperature. Sea cucumbers with similar size (4.02 ± 0.11 g) were cultured for 8 weeks at 14 °C, 18 °C, 22 °C and 26 °C, respectively. At the end of the experiment, the specific growth rate (SGR) and the profiles of FAs in neutral lipids and phospholipids of the juvenile sea cucumbers cultured at different temperatures were determined. The SGRs of the sea cucumbers cultured at 26 °C significantly decreased 46.3% compared to thos cultured at 18 °C. Regression analysis showed that the SGR-temperature (T) relationship can be expressed as $SGR = -0.0073\ T^2 + 0.255\ T - 1.0231$ ($R^2 = 0.9936$) and the highest SGR was predicted at 17.5 °C. For the neutral lipids, the sum of saturated FAs (SFAs), monounsaturated FAs (MUFAs) or polyunsaturated FAs (PUFAs) of the sea cucumbers that were cultured at the water temperature from 18 °C – 26 °C did not change significantly, indicating the insensitivity of FA profiles for the neutral lipids of sea cucumbers in response to increasing water temperature. For phospholipids, the sum of PUFAs in the sea cucumbers dramatically decreased with the gradually increased water temperature. The sum of SFAs and MUFAs of sea cucumbers, however, increased with the gradually elevated water temperature. In particular, the contents of highly unsaturated fatty acids (HUFAs), including eicosapentaenoic acid (EPA, 20:5$n-3$) and docosahexaenoic acid (DHA), in the phospholipids of the sea cucumbers decreased 37.2% and 26.1%, respectively, when the water temperature increased from 14 °C to 26 °C. In summary, the sea cucumbers *A. japonicus* can regulate the FA compositions, especially the contents of EPA and DHA, in the phospholipids so as to adapt to varied water temperature.

1. Introduction

Water temperature is one of the most important ecological factors that affect the feeding behavior, metabolism, growth and even survival of poikilothermic sea cucumbers (Dong et al., 2008a; Ji et al., 2008, 2011; Zamora and Jeffs, 2012; Shao et al., 2015; Wang et al., 2015). It is hence necessary to determine suitable water temperature for growth and fatty acid (FA) quality of sea cucumbers due to the huge market demands and wide culture of sea cucumbers in northern China and other Asian areas (Yang et al., 2006; Yuan et al., 2006; Han et al., 2016; Yu et al., 2016). Numerous studies have been conducted focusing on the thermal physiology of sea cucumber (Yang et al., 2006; Dong et al., 2008b; Ji et al., 2008; Shao et al., 2015).

To maintain physiological homeostasis under temperature fluctuations, aquatic animals have developed specific adaptative mechanisms, either behavioral, physiological, or biochemical. Changes in FA profiles of aquatic animals which were exposed to varied water temperature have been found in many previous studies (Hsieh and Kuo, 2005; Alhazzaa et al., 2013). Fatty acids, including combined FAs in neutral lipid and phospholipids and free FAs, are indispensable to living organisms, for the reasons of contributing energy resources, being essential nutrients for survival and growth, and playing important roles in the structure and function of cell membranes (Alfaro et al., 2006; Wang et al., 2007). Short-term exposure, over a period of days or a few weeks, of poikilotherm to sub-optimal temperature can change the unsaturation levels of lipids (Hazel and Landrey, 1988; Zehmer and Hazel, 2005). Alteration in the compositions of unsaturated FAs to maintain cell membrane fluidity had been shown to be an important adaptive process for aquatic animals during temperature fluctuations (Hsieh and Kuo, 2005; Alhazzaa et al., 2013). Desaturation of FAs can maintain the fluidity of cell membrane, and

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http://dx.doi.org/10.1016/j.jtherbio.2016.07.011
0306-4565/

consequently compensate the rigidification of lipids in cells exposed to varied water temperature and control the ratio of unsaturated: saturated FAs which was required for membrane lipid homeostasis (Mansilla et al., 2008; Miller et al., 2010). Hence, understanding mechanistic and compositional responses of storage and membrane lipids to the offset temperature will provide new insights into the nature of acclimatization for farmed sea cucumbers. Changes in FA profiles of neutral lipids and phospholipids of juvenile sea cucumber *Apostichopus japonicus* (Selenka) in response to varied water temperature, however, remain poorly known to the present.

The present study was conducted to determine the effects of varied water temperature on FA profiles in neutral lipid and phospholipid of juvenile sea cucumbers *Apostichopus japonicus* (Selenka), aiming to evaluate the participation of FAs in the adaptation to water temperature change.

2. Materials and methods

2.1. Experimental animals and diets

Experimental sea cucumbers were collected from a local sea cucumber rearing farm in Qingdao, China. Prior to the start of the experiment, sea cucumbers were reared in plastic tanks at 18 °C for two weeks to adapt to the experimental conditions and feeds. Ingredients and nutrient composition of the experimental diets are given in Table 1. All ingredients were ground into fine powder through a 200 mm sieve and thoroughly blended. Pellets were made automatically by pellet-making machine (Weihai, Shandong province, China) and dried for about 10 h in a ventilated oven at 40 °C. Feeds were packed in double plastic bags and stored at −20 until use (Table 2).

2.2. Experimental procedure and sample collection

After acclimation, the sea cucumbers were fasted for 24 h. Then 160 sea cucumber individuals with similar size (4.02 ± 0.11 g) were selected and randomly distributed into 20 tanks (45 × 25 × 30 cm). For each of the 4 experimental temperatures (14 °C, 18 °C, 22 °C and 26 °C), 5 tanks of sea cucumbers were kept as replicates. Water temperature was controlled by the automatic temperature controller which was equipped with heater and chiller, and the temperature changed to the test temperatures by 1 °C per day. The sea cucumbers were fed with the ration of 5% body wet weight once a day at 4:00 pm. During the experiment, aeration was provided continuously, and half water of each tank was exchanged by fresh equi-temperature seawater every day at 10:00 am. Seawater temperature was kept by an autoregulation induction heater. Salinity 29–30, pH 7.9–8.7, and dissolved oxygen > 5.0 mg L^{-1}.

The experiment lasted for 8 weeks. At the termination of the experiment, the sea cucumbers were fasted for 24 h, and then the total number and body weight of sea cucumbers in each tank were measured. After weighed, all sea cucumber individuals in each tank were dissected on ice and the separated body wall were put into plastic bags. All samples were frozen in liquid nitrogen and stored at −80 °C immediately for further analysis.

2.3. Calculation of growth

The specific growth rate (SGR) and the thermal growth coefficient (TGC) were calculated as follows:

Table 1
Proximate composition of trial diets for *A. japonicus* (Dry matter basis).

Ingredients	(%)
Fish meal[a]	15
Sargassum thunbergii[a]	43
Vitamin premix[b]	0.5
Mineral premix[c]	0.5
Fish oil	0.5
Soybean oil	0.5
Sea mud[a]	40
Proximate composition (%)[d]	
Moisture	9.01
Crude protein	18.9
Crude lipid	2.10
Ash	34.7

[a] *Sargassum thunbergii* (dry matter, %): crude protein 19.4, crude lipid 2.00; fish meal (dry matter, %): protein 70.1, crude lipid 8.06; sea mud (dry matter, %): protein 2.74, crude lipid 0.90. These ingredients were obtained from Great seven Bio-Tech (Qingdao, China).

[b] Vitamin premix contained the following amount which were diluted in cellulose (g kg^{-1}premix): L-ascorbic acid, 100; DL-a-tocopheryl acetate, 2; thiamin hydrochloride, 8; riboflavin, 10; pyridoxine hydrochloride, 15; niacin, 45; Ca-D-pantothenate, 18; myo-inositol, 80; D-biotin, 0.3; folic acid, 1.5; menadione, 4; retinyl acetate, 3.2; cholecalciferol, 1; cyanocobalamin, 0.004; ethoxyquin 16.

[c] Mineral premix contained the following ingredients which were diluted in zeolite (g kg^{-1} premix): $MgSO_4$ $7H_2O$, 80.5; Ferric citrate, 16; $ZnSO_4 H_2O$, 9; $CuSO_4$ $5H_2O$, 3; $AlCl_3$ $6H_2O$, 6; KIO_3, 0.04; $MnSO_4$ H_2O, 2; $CoCl_2$ $6H_2O$, 0.04.

[d] The proximate compositions of diets were determined following the method of AOAC (1995).

$$\mathrm{SGR}\left(\%.\ \mathrm{d}^{-1}\right) = 100 \times \left(\mathrm{LnW_t} - \mathrm{LnW_0}\right) / t,\ \mathrm{TGC}\left(\sqrt[3]{g}\ (^{\circ}C.\mathrm{d})^{-1}\right) = \left[\left(\sqrt[3]{W_t} - \sqrt[3]{W_0}\right)/(T \times t)\right] \times 1000,$$

Where W_0 and W_t were initial and final body weight (g) of sea cucumber, respectively; T was the average temperature (°C) and t (days) was duration of experiment. Body weight of sea cucumbers in each tank was expressed as average value of 20 sea cucumbers.

2.4. Analysis of fatty acid profiles

The total lipids in the body wall of the sea cucumbers were extracted with chloroform-methanol mixture (2:1, v/v) according to the method of Folch et al. (1957). According to the method of Lou et al. (2012), the neutral lipids, glycolipids and phospholipids were fractionated on a Bond Elut SI (3 mL, 500 mg, sigma) using chloroform, acetone and methanol, respectively, and sequentially as eluents.

Fatty acid profiles in the separated neutral lipids and phospholipids of the sea cucumbers were analyzed using the methods that were described in Yu et al. (2015). Briefly, fatty acid methyl esters (FAMEs) were prepared by esterification using 2% sulfuric acid methanol (Gao et al., 2006). FAMEs were separated and quantified by means of a gas chromatograph (Shimadzu GC-2010, Shimadzu Scientific Instruments, Columbia, MD, USA) equipped with a RTX-wax plus capillary column (30 m long, 0.25 mm internal diameter, 0.25 μm thickness; Phenomenex, Torrance, CA, USA) and flame ionization detector (GC-FID). Hydrogen was used as carrier gas (4 mL min^{-1}), and the injector and detector temperatures were 250 and 260 °C, respectively. Each of the specific FAME peak was identified by the retention time with reference to the known standard (Supelco, Inc., Bellefonte, Pennsylvania, USA). The relative amount of each fatty acid in the neutral lipids or phospholipids was expressed as the percentage of the specific fatty acid in the sum of total neutral lipids or phospholipids.