Aquaculture International (2021) 29:2403–2414
https://doi.org/10.1007/s10499-021-00756-9

Effect of different diets on growth of the sub-adult sea cucumber *Holothuria scabra*

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Received: 5 February 2021 / Accepted: 18 July 2021/ Published online: 28 July 2021

Abstract
In recent years, the demand for commercial sea cucumber diet increased due to the expansion of sea cucumber farming. In this study, we investigated alternative sources other than the use of macroalgal powder for formulating sea cucumber feed. Specific growth rate (SGR), total weight gain, and daily weight gain were determined for sea cucumber (45 individuals, initial weight: 51.08±3.29g) fed with three diets containing different amounts of nutrients (crude protein: crude fat): formulated diet- C (14.5: 4.3%), sea cucumber commercial diet- A (7.5: 0.8%), and dried shrimp waste diet- B (5.5: 0.6%). Sediment redox oxic-anoxic interface regime was evaluated referring to the color change between oxic (light brown) and anoxic (grey) from total sediment sample core. The animals fed with shrimp waste diet showed negative growth and rapid reduction in weight gain and was not suitable for intensive sea cucumber farming. The sea cucumber fed with formulated diet showed faster growth, highest SGR, and daily weight gain when compared to sea cucumber fed on commercial diet. The formulated diet showed the highest impact in accumulating the anoxic sediment condition followed by commercial diet and dried shrimp waste. The formulated diet seems to be a promising alternative source which needs more focus on the nutritional value in order to balance the impact on sediment quality.

Keywords Aquaculture; · Sea cucumber; · *Holothuria scabra*; · Formulated feed; · Intensive farming

Handling Editor: Gavin Burnell

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Introduction

Holothuria scabra, which is known as sand fish, is one of the most valued tropical sea cucumber products in the market of the fish food industry, directing an average market price of US$ 303 kg^{-1} dry weight and reaching high up to US$ 1668 kg^{-1} for first grade (Purcell 2014; Robinson et al. 2015). The global aquaculture production of sea cucumber was 204,704 tons in 2016. The overall production of natural fisheries in 2007 from Asia and the Pacific regions was in the range of 20,000 to 40,000 tons per year (Toral-Granda et al. 2008; FAO 2021). Overfishing has led to being listed as endangered in the IUCN Red list (Purcell et al. 2014). The decline of natural stocks of holothurians in marine habitats leads to the initialization of commercial aquaculture programs tropical sea cucumbers (Battaglene et al. 1999; Conand 2004; Pitt and Nguyen 2004; Agudo 2006). In recent years, farming of sea cucumbers has expanded rapidly becoming an important aquaculture sector (Sun et al. 2015). The common farming methods such as land-based ponds, pen culture, and semi-intensive culture have been developed for increasing the production of sea cucumbers (Chang 2003; Kang et al. 2003; Yuan et al. 2006).

Holothuria is deposit-feeding which usually eat the organic matter associated with the sediment such as bacteria, cyanophyceans, and foraminiferans (Wiedemeyer 1992; Mercier et al. 2000). Natural food is a limiting factor for the growth of sea cucumber in culture systems because of high stocking density, and artificial diet plays an important role in increasing the production output (Qin et al. 2009). Knowledge on the feeding behavior and nutritional requirements are essential for successful farming of sea cucumber. Such information could be useful for designing the feeding regimes in order to minimize the wastage (Slater et al. 2009; Sun et al. 2015). For sea cucumbers, little is known about the role of formulated feeds on the growth induction and health (Yuan et al. 2006; Zhou et al. 2006; Slater et al. 2009). Many previous studies on feed composition, nutritional requirement and digestion capability were conducted using larvae and juveniles of sea cucumber (Fu et al. 2005; Okorie et al. 2008; Slater et al. 2009; Li et al. 2009; Seo and Lee 2011). However, few studies on these aspects were discussed farming sea cucumber *H. scabra* with supplemental feed. For instance, Giraspy and Ivy (2008) has evaluated the commercially available supplemental feeds such as Algamac 2000, Algamac protein plus, *Spirulina* and *Dunaliella* gold for sea cucumber farming.

In formulated feeds, macroalgal powder and sea mud are used as main ingredients because the sea mud and algal detritus are the most important food sources for sea cucumbers (Liu et al. 2010). Juveniles of the sea cucumbers that were fed with sea mud and macroalgal powder showed higher growth rates than other feeds (Battaglene et al. 1999; Liu et al. 2010; Zhu et al. 2007). Macroalgal powder is an essential feed ingredient for cultured sea cucumber. The increased demand of algal resources with rapid expansion of sea cucumber commercial farming leads to severe damage and shortage of materials as some algal species is not produced commercially which led in getting it expensive (Xia et al. 2012; Anisuzzaman et al. 2017). Hence, it is important to formulate alternative feeds using low cost ingredients for farming of sea cucumber *H. scabra* at commercial scale. The present study was conducted to evaluate three types of diets for growing sea cucumber and their impact on sediment quality. The results of this study will be useful for the formulation of artificial diets for sea cucumber farming.

Material and methods

Animal transportation and acclimatization

This study was carried out in the hatchery of the Faculty of Marine Sciences, King Abdulaziz University, Jeddah, Saudi Arabia under controlled condition. Sea cucumbers used in this study were collected from nursery ponds of National Aquaculture Group, Saudi Arabia. For transporting to the experimental site, four crates (L:60 × W:70 × H:16 cm) were lined with cloth sheet and stacked in the fiberglass tub of 300L seawater capacity (40 psu salinity). Each layer was stocked with 50 animals (Fig. 1). Water was enriched with pure oxygen and cooled with ice bags to maintain temperature at 24°C. Animals were distributed into 3 circular fiberglass tanks (Bottom dim: 1.9 m * H: 1.0 m) and bottom was covered with dune sand (5 cm depth of substrate) for 7-day acclimation. During the acclimatization period, continuous over-flow water exchange was carried out and sea cucumbers were fed once per day with the commercial diet at the ratio of 2% of total wet weight (Robinson et al. 2016).

Experimental feed preparation

The experiment was designated for testing three diets: diet A- Sea cucumber commercial compound feed GO (sensory characteristic: dark brown color, 4 mm pellet, 9.8% moisture) imported from Laizhou Baishengd Technology Co., Ltd, China which is originally used for the commercial cultivation of sea cucumber *A. japonicas*. Diet-B was dried shrimp waste solids

Fig. 1 Transportation container of sea cucumber. (Container water volume 300 L and crate size L:70 × W:60 × H:16 cm)

which apparently consisted of uneaten feed and debris collected through 1000-micron trap net from whiteleg shrimp (*Litopenaues vannamei*) culture ponds. The waste was sun-dried for a month then ground to powder and sieved with 0.2 micron mesh. Diet-C was a formulated diet consisted of a mixture of 15% wheat bran, 5% fish meal, 4.75% soybean, 10% barley, 0.25% vitamins and minerals, and 15% fish feed powder. The raw materials of diet-C were powdered and sieved with 250 micron mesh, mixed with 10% molasses then the mash was included to 40% sea mud and mixed properly. The sea mud which mainly consists of sandy clay (grain size 0.04~0.06 mm and OM% 0.82±0.06) was collected from Al-Shoiba lagoon area (N20°54 ′12′E39°22′12) and then sundried before using. All types of diets were soaked for 12 h in water previous to feeding. The proximate composition of the diets used in this study was analyzed according to AOAC International, official methods of analysis (Horwitz 2000).

Experimental design

After the acclimation period, sea cucumbers were suspended in a soft mesh bag inside the acclimation tank for 24 h to evacuate guts for weighing. Sea cucumbers with an initial average body weight (ABW) of 51.08±3.29g (mean± SD) were selected from respective acclimatized animals and distributed into 9 fiberglass tanks of 500L (1m^2 bottom area X H: 0.8m) at a stocking density of 5 animals per tank (250 g m^2) to form 3 groups (in triplicates) as shown in Fig. 2. The experimental tanks were filled with 5 cm sand dune substrate (grain size 0.15~0.25 mm and OM% 0.27±0.04) which was collected from Al-laith highway (N20°19 ′50′E40°02′43). The randomized block design was used for the experiment. The three

Fig. 2 Setup used for the feeding experiment. (Tank size, bottom di:1m^2 × H:0.8m)

experimental groups were fed twice daily with respective diet A, B and C at the ratio of 2% which was divided into two portions at 8:00 am and 8:00 pm (Xia et al. 2017).

Physical parameter and water exchange

Water exchange was done daily at 100% in two times 7:00 am and 7:00 pm using a filter bag (10 micron). The parameters of water such as dissolved oxygen, temperature, and pH were recorded daily at 7:00 am and 7:00 before carrying out the water exchange using a multiparameter probe YSI Pro 1020 (Xylem Analytics, Gulf Advanced Control System Est., Rastanura, KSA). Aeration was provided continuously to maintain DO above 5.0 mg L^{-1} in the tanks, salinity was 40 psu and the photoperiod of 12L:12D was maintained throughout the experiment using fluorescent light. The water quality parameters of the experimental tanks during the study period are presented in supplementary figures (supplementary figures 1-3).

Sample collection

The experiment ran for 60-day period. All sea cucumbers from each replicate were gut evacuated for 24 h and average body weight (ABW) was reassessed every 29 days using an electronic digital balance (resolution=0.01g). Average body weight data was used to calculate specific growth rate (SGR), weight gain and weight increment. Sediment quality was evaluated weekly by measuring the oxic-anoxic interface of tanks bottom sediment using a modified syringe for collecting sediment core from base of the tank to surface. Depth of sediment oxic-anoxic interface was measured in centimeter (using a standard plastic ruler) referring to the color change between oxic (light brown/yellow) and anoxic (grey) and the values were converted to percentage from total sediment depth of the sample as shown in Fig 3. Sea cucumber feces were collected daily from each group across the experimental period and pooled samples were kept frozen for analysis of proximate composition. At the end of the experiment, animals of each group were collected and pooled as one sample and kept in frozen condition for analyzing proximate composition (AOAC Official Methods 17th Edition, Horwitz 2000).

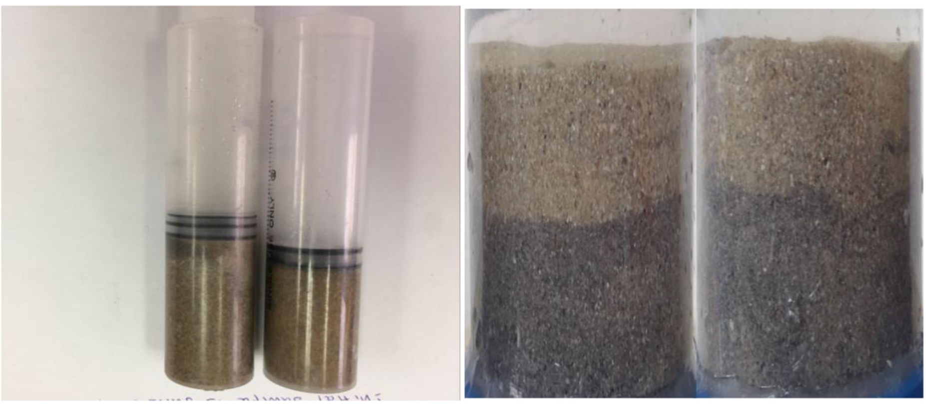

Fig. 3 Sediment core used for the measurement of oxic-anoxic sediment interface in the culture tanks

Data analysis

Two-way ANOVA (analysis of variance) was used to find out the variations of average body weight and monthly weight gain of *H. scabra* treated with different diets. The diet type and culture days were used as factors for the ANOVA. Furthermore, one-way ANOVA was carried out for daily weight gain and SGR data. The data were checked for homogeneity of variance using Cochran C test. Post hoc Tukey test was used if the significant variation was observed for the factor diet type in ANOVA. For all statistical tests, $P<0.05$ was considered significant.

Results

The proximate composition of tested diets revealed the highest crude protein for diet-C 14.5%, and 7.5% for diet-A and 5.5% for diet-B while crude fat recorded highest range with 4.3%, 0.8% and 0.6% for diet-C, diet-A and diet-B, respectively (Table 1). The growth performance of sea cucumber during the culture period is presented in Table 2. There was a significant difference in overall body weight of *H. scabra* fed on the different tested diets (Two-way ANOVA, Table 3). Post hoc Tukey test results further confirmed that all the treatment diets significantly varied each other in terms of weight gain in sea cucumber after 60 days (Supplementary Table 1). The highest average body weight observed after 60 days of culture were 110.4 g for the formulated diet, 64.54 g for commercial diet and 37.45 g for shrimp waste (Table 2). The results showed that the highest weight gain was 59.96 g for the formulated diet with a final weight increment of 119%, followed by the commercial diet with 14.78 g weight gain and 31% as total weight increment, whereas the shrimp waste recoded a negative growth of -15.56 g and 29% reductions in weight during the culture period.

The average daily weight gain recorded was 1, 0.2 and -0.26 g for formulated diet, commercial diet and shrimp waste, respectively. The daily weight gain differed significantly between the different feed treatment groups (F=219.8; df= 2.6; $P<0.001$, for Tukey test results see: Supplementary Table 2). The SGR of experimental groups achieved 1.30 and 0.44 % d^{-1} in diet-C and diet-A respectively. The SGR was -0.58% d^{-1} in dried shrimp waste (Diet B) treated group. One-way ANOVA revealed a significant variation in the SGR of sea cucumbers fed with different diets (F=97.50; df= 2, 6; $P<0.001$). Pair-wise comparisons between the SGR

Table 1 Analysis of proximate composition of experimental diets. Values are on wet basis (n=1)

Parameter	Diet A	Diet B	Diet C
Moisture (%)	9.8	4.5	8.4
Crude Protein (%)	7.5	5.5	14.5
Nitrogen (%)	1.2	0.9	2.3
Crude Fat (%)	0.8	0.6	4.3
Crude Ash (%)	57.2	73.8	38.0
Crude Fibre (%)	4.4	3.5	2.2
Nitrogen Free Extract-NFE (%)*	20.3	12.1	32.6
Carbohydrate (%)*	24.7	15.6	34.8
Organic Carbon (%)*	19.18	12.6	31.16
Gross Energy (kcal kg^{-1})*	1360	898	2359

* Nitrogen free extract, carbohydrate, organic carbon, and gross energy was given by calculation

Table 2 Growth performance (mean ± SD, n=3) of sea cucumbers fed with three different diets

Growth parameters	Diet treatment groups		
	Diet A	Diet B	Diet C
Overall summary			
Initial weight (g)	49.76±4.94[a]	53.02±2.60[a]	50.44±1.86[a]
Final weight (g)	64.54±1.25	37.45±3.23	110.41±5.95
Final weight gain (g)	14.78±5.72	-15.56±5.15	59.96±5.57
Total weight increment (%)	31±15	-29±9	119±12
Growth (g day^{-1})	0.20±0.10[a]	-0.26±0.09[b]	1.00±0.09[c]
Specific growth rate (% day^{-1})	0.44±0.19[a]	-0.58±0.21[b]	1.30±0.09[c]
30 days			
Average body weight (g)	58.16±4.61[a]	51.80±4.25[a]	85.19±4.06[c]
Monthly weight gain (g)	8.40±1.05[a]	-1.22±5.76[a]	34.74±4.14[c]
Monthly weight increment (%)	17±3	-2±11	69±9
60 days			
Average body weight (g)	64.54±1.25[a]	37.45±3.23[b]	110.41±5.95[c]
Monthly weight gain (g)	6.38±5.20[a]	-14.35±1.24[b]	25.22±2.08[c]
Monthly weight increment (%)	11±10	-28±1	30±1

All animals were alive at the end of the experiment

Data having different superscripts in same row are significantly different between each other (Tukey test: $P<0.05$)

of sea cucumbers showed significant variations between the treatment groups (Supplementary Table 2).

Two-way ANOVA results revealed significant variations in monthly weight gain between the diet treatments and days of culture (Table 3). While, Tukey test results showed significant variations in monthly weight gain between three different diet treated sea cucumbers after 60 days, diet-A and diet-B treated groups did not show significant variation in monthly weight gain after 30 days (Supplementary Table 3). The formulated diet gained 34.74g in weight during the first 30 days with 69% increment which declined to 25.22g weight gain at a reduction of 39% in weight gain during the experiment period of 30-60 days. The monthly weight gain recorded in the commercial diet treatment after 30 days was 8.40 g with 17% increment which declined to 6.38 g and 11% at 60 days of culture. Whereas the shrimp waste showed a significant negative growth which recorded -1.22 g on 30 days of culture with 2% reduction in weight which further decreased to 28% (-14.35 g) at 60 days.

The proximate composition of the sea cucumber body and feces are presented in Table 4. The obtained crude protein of sea cucumber (body composition; feces) was (6.5; 0.26%), (5.9; 0.18%) and (3.9; <0.1%) for diet-A, diet-C and diet-B, respectively whereas the obtained crude fat recorded was (0.49; not detected %), (0.35; 0.14%) and (0.29; 0.11%) respectively for diet-

Table 3 Two-way ANOVA results ($P<0.05$= significant) for the average body weight and monthly weight gain of sea cucumbers treated with three types of diets

	Average body weight				Monthly weight gain			
	Df	*MS*	*F*	*p*	*Df*	*MS*	*F*	*P*
Days	2	926.3	60.50	0.000	1	304.46	21.65	0.000
Diet	2	2848.9	186.10	0.000	2	2167.03	154.12	0.000
Days*Diet	4	1091.4	71.29	0.000	2	48.13	3.42	0.066
Error	18	15.3			12	14.06		

B, diet-A and diet-C. The sediment anoxic condition during the culture period is presented in Fig. 4. The highest anoxic sediment condition at the week 8 of experiment was observed in tanks fed on formulated diet with 60% anoxic sediment condition. The commercial diet maintained a moderate good sediment quality and recoded 35% of anoxic sediment condition whereas the shrimp waste showed the lowest anoxic sediment condition of 15%. No mortality was observed in the experimental groups.

Discussion

The results of this study indicated that the weight gain or SGR of sea cucumbers were significantly influenced by the diets. Furthermore, the proximate composition analysis of the diets indicated that, the protein and other nutrients content of the experimental diets were not similar (Table 1). Zhu et al. (2005) revealed the optimum protein and lipid requirement for the growth of sea cucumber juvenile *Stichopus japonicus* as 18–24% and 5% respectively. The formulated feed prepared in this study contained the protein and lipid content of 14.5 and 4.3% respectively, which was slightly below the requirements reported by Zhu et al. (2005). Furthermore, sea cucumbers fed with dried shrimp solid waste exhibited a reduction in growth (negative growth). shrimp waste is a low-energy feed, and the quantity of energy consumed by sea cucumbers was insufficient to compensate for energy losses in feces, excretion and respiration, thus they had to use energy stored in the body before and this could be one of the possible reasons for the negative growth. Another reason, feces sun-drying process for a month might have removed the beneficial bacteria and nutrients such as vitamins and fatty acids. According to Yingst (1976) that many deposit feeding holothurians have low cellulose activity in guts and do not seem to absorb nutritional stuff before it is breakdown by bacteria and fungi. Chen et al. (2015) claimed that sea cucumber can hardly absorb the dried shrimp waste. The results further confirmed that using dried shrimp waste solid alone as a feed is not suitable for feeding sea cucumber under intensive farming. This observation agreed with Yuan et al. (2006) who found that using a diet of sole dried bivalve feces on sea cucumber *A. japonicas* resulted on a negative growth and recorded SGR of -0.66 % d^{-1} (wet weight 30g) whereas mixed dried bivalve feces with powdered algae yielded a promising result, which directed him to conclude that the sole dried feces of bivalve is not suitable feed for intensive sea cucumber farming. The current study disagreed with the result of Chen et al. (2015) who reported that a diet of sole dried pure shrimp waste led to increase in total body weight,

Table 4 Analysis of proximate composition sea cucumbers and feces of treatment groups

Nutrient content	Diet treatment groups			
		Diet A	Diet B	Diet C
Moisture (%)	Body composition	75.75	74.52	85.03
	Feces	20.9	21.3	25.9
Crude Protein (%)	Body composition	6.5	3.9	5.9
	Faces	0.26	<0.1	0.18
Crude Fat (%)	Body composition	0.35	0.49	0.29
	Feces	0.14	ND	0.11
Crude Ash (%)	Body composition	15.43	19.18	8.88
	Feces	99.1	99.2	99.3

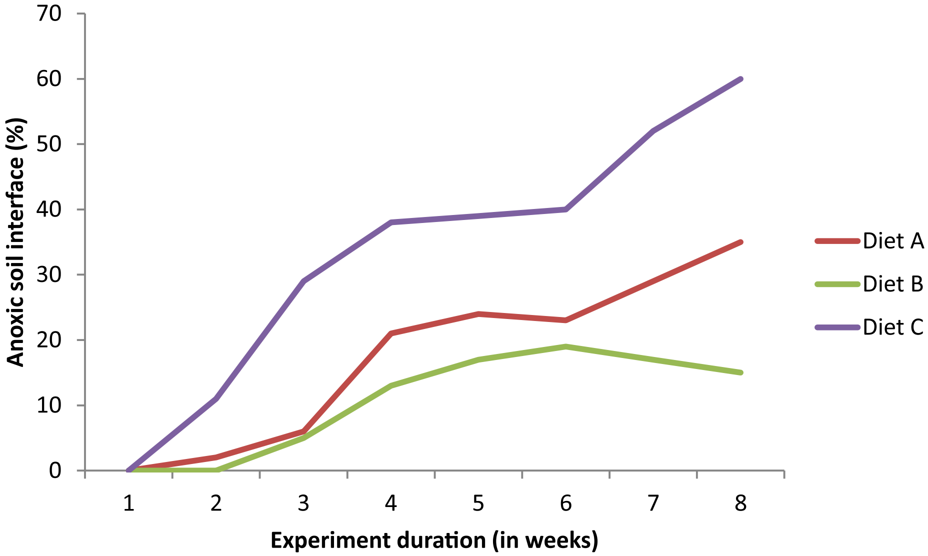

Fig. 4 Sediment anoxic layer (%) measured from the three experimental group tanks

recorded SGR of 0.29 % d^{-1} in sea cucumber *Stichopus monotuberculatus*, even though it recorded the lowest growth when compared to mixed diets at different portions of shrimp waste with powdered algae and mud. even though shrimp waste is hardly absorbed by the Sea cucumber. It is worth mentioning that the dietary protein of sole shrimp waste reported by Chen et al. (2015) was 13.98% whereas the crude protein in dried shrimp waste of the current study is 5.5% and this could be the reason of recorded negative growth.

The formulated diet (diet-C) showed a promising result for farming of sub-adult *H. scabra* when compared to the commercial diet (diet-A). A previous study by Yuan et al. (2006) showed the SGR of 1.31% to 2.13% d^{-1} for the sea cucumber *A. japonicas* fed on different formulated diets containing a protein range from 1.4% to 11.03%. Another study by Chen et al. (2015) achieved SGR between 0.3 and 1.2 % d^{-1} in sea cucumber *S. monotuberculatus* fed with different protein diets range from 13.98 to 21.54%. Furthermore, unlike that using the commercial compound diet during the acclimation period affected results of the current study as it is the same diet used from where the sea cucumbers were brought. Also, the animals were starved in the acclimation tank for 24 h before distribution into the experimental tanks where only the experimental diet was introduced.

The current study revealed that Diet A had a much lower protein level than diet C, but produced a higher animal protein level. A decrease in protein content of the sea cucumbers fed with diet B was visible due to the low crude protein content in this diet. The higher protein content of the body might be due to the consumption of high protein content diet by the sea cucumber. Similar result of increase in protein content due to the consumption of high protein diet was reported by Chen et al. (2015). In this study, the nutritional content of sea cucumbers treated with different diets was higher in the body than in excreted feces and this is in agreement with the result reported by Zhou et al. (2006) and Chen et al. (2015). Diet B produced the highest body crude fat level among the three diets. This may be due to the low energy content of this diet which is not enough to meet the metabolic requirements, so the sea cucumbers treated with shrimp waste had to deplete the deposited energy in the body and this is similar to findings reported by Yuan et al. (2006).

Sea cucumbers are deposit feeders in the marine ecosystems and eat the organic-rich sediment as their diet (Klinger and Johnson 1998). Hence, it is important to assess the quality of the sediment in the culture systems because it directly influences the growth of sea cucumbers. In this study, the experimental tanks had the same anoxic sediment condition at beginning of the experiment. However, the anoxic sediment conditions varied between the different diet treatment tanks over the treatment duration. Hydrogen sulfide (Un-ionized H2S) is very toxic to aquatic organisms, even at natural levels (Knezovich et al. 1996). The stratified oxic-anoxic sediment regime has more abundant and stabilize microbial communities which supports the biomass increase (Robinson et al. 2016). It was reported that rearing of sea cucumber on full oxic sediments led to stunned growth and gained a lower biomass in comparison to those reared in stratified oxic-anoxic sediments (Robinson et al. 2015). The result of current study indicates that the anoxic level of sediment condition increased over the time and this might be the reason of the observed decline in monthly weight gain among all experimental diets and this might be because the limitation of grazing area as sea cucumbers were observed avoiding the black sediment area. The findings agreed with Robinson et al. (2016) who reported that the redox regime is a principal factor influencing growth of sea cucumber, biomass yield, behaviour and biochemical utilisation. Even though the growth rate of sea cucumber was positive throughout the duration of the study; however, the growth rate declined over time.

In conclusion, this study showed that sea cucumbers exhibited the highest weight gain when they fed on formulated diet compared to commercial diet which indicates the potential of the formulating diet for sea cucumber semi-intensive farming. However, the formulated diet impacted negatively on the sediment quality. The cumulative organic content should be considered for the use of the formulated diet in order to balance the oxic-anoxic condition of the sediment. Further studies on maintenance of water and sediment quality of the ponds when using the formulated feed may improve its capability in commercial applications.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10499-021-00756-9.

Acknowledgements We thank the Managing Director of National Aquaculture Group for providing sea cucumbers and technical support for this study. We are also thankful to the Dean, Faculty of Marine Sciences, King Abdulaziz University, for the facilities provided.

Author contribution Conceptualization: Mohammed Broom, Sathianeson Satheesh; Methodology: Mohammed Broom, Mamdouh Al-Harbi; Formal analysis and Investigation: Mohammed Broom, Sathianeson Satheesh; Writing-original draft preparation: Mohammed Broom, Mohamed Hosny Gabr; Writing-review and editing: Sathianeson Satheesh; Resources: Mamdouh Al-Harbi, Mohamed Hosny Gabr; Supervision: Sathianeson Satheesh. All the authors approved the final draft.

Data availability The data generated in this study are available from the corresponding author on reasonable request.

Code availability Not applicable

Declarations

Ethics approval The animals used for this study were sourced from the fish farm. The authors followed the international and institutional guidelines for handling the animals for the experiment.

Consent to participate Not applicable

Consent for publication All the authors approved the final draft for publication

Conflict of interest The authors declare no competing interests.

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