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Effect of Shrimp (*Litopenaeus vannamei*) Farming Waste on the Growth, Digestion, Ammonium-Nitrogen Excretion of Sea Cucumber (*Stichopus monotuberculatus*)

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Abstract In this study, specific growth rate (SGR), ingestion rate (IR), food conversion ratio (FCR), apparent digestion ratio (ADR) and ammonium-nitrogen excretion were determined for sea cucumber (*Stichopus monotuberculatus*) reared in plastic containers (70 L; 4 containers each diet treatment). Sea cucumbers were fed with five diets containing different amounts of farming waste from shrimp (*Litopenaeus vannamei*) (100%, 75%, 50%, 25% and 0) and a formulated compound (20% sea mud and 80% powdered algae). Sea cucumbers grew faster when they were fed with diet D (25% shrimp waste and 75% formulated compound) than those fed with other diets. Although IR value of sea cucumber fed with diet A (shrimp waste) was higher than those fed with other diets, both the lowest SGR and the highest FCR occurred in this diet group. The highest and the lowest ADR occurred in diet E (formulated compound) and diet A group, respectively, and the same to ammonium-nitrogen excretion. The contents of crude protein, crude lipid and total organic matter (TOM) in feces decreased in comparison with corresponding diets. In the feces from different diet treatments, the contents of crude protein and TOM increased gradually as the contents of crude protein and TOM in diets increased, while crude lipid content decreased gradually as the crude lipid content in diets increased.

Key words sea cucumber; *Stichopus monotuberculatus*; shrimp; *Litopenaeus vannamei*; growth; digestion; ammonium-nitrogen excretion

1 Introduction

The white shrimp, *Litopenaeus vannamei*, is a predominant penaeid species, which is currently cultured worldwide (Frias-Espericueta *et al.*, 2001; McGraw *et al.*, 2002; Saoud *et al.*, 2003; Cheng *et al.*, 2006). The culture of *L. vannamei* has been widespread in Asia (Andriantahina *et al.*, 2012). Shrimp aquaculture industry has produced a large amount of farming waste. Generally speaking, farming waste from shrimp is a mixture mainly composed of effluent containing high concentrations of nitrogen and phosphorus, and sediment containing uneaten feeds, shrimp excrement and biological remains. Additionally, it was reported that the shrimp waste was helpful for the rapid growth of phytoplankton (Tookwina and Songsangjinda, 1999). Moreover, phytoplankton in shrimp ponds mostly consisted of a variety of algae and some zooplankton existing universally in shrimp aquaculture water body (Tookwina and Songsangjinda, 1999; Maristela *et al.*, 2008). Thus, it seemed that shrimp waste was mainly composed of uneaten feeds, feces, various algae debris and zooplankton remains after it was dried. In recent years, farming waste from shrimp aquaculture industry has led to serious coastal ecosystem pollution (Sansanayuth *et al.*, 1996; Páez-Osuna *et al.*, 1998; Páez-Osuna, 2001; Burford and Williams, 2001; Anh *et al.*, 2010). Therefore, dealing with shrimp farming waste is a key measure to alleviate the pollution (Chin and Ong, 1994).

As a commercially valuable species, sea cucumber (*Stichopus monotuberculatus*) is distributed widely (Fan *et al.*, 2012; Massin *et al.*, 2002; Rowe and Gates, 2004; Byrne *et al.*, 2010). At present, the research on this species is still at the starting stage. Hu *et al.* (2010) studied the larval development and juvenile growth of *Stichopus* sp. (curry fish), and it was identified as *S. monotuberculatus* later (Fan *et al.*, 2012). The development of sea cucumber aquaculture industry needs knowledge of dietary requirements. However, up to now, less data regarding the feeding behavior, nutrition requirements and artificial feeds of this species are available. This has hindered the development of *S. monotuberculatus* aquaculture industry.

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It has been demonstrated that sea cucumber can ingest uneaten feeds and feces of marine animals, even their own feces (Hauksson, 1979; Tiensongrusmee and Pontjoprawiro, 1988; Goshima *et al.*, 1994; Ramofafia *et al.*, 1997). In previous studies, as a feasible and cost-effective method, polyculture was often used to rear both species to larger sizes and reduce the pollution from aquaculture industry due to above feeding habit of sea cucumber (Ahlgren, 1998; Kang *et al.*, 2003; Zhou *et al.*, 2006; Slater and Carton, 2007; Paltzat *et al.*, 2008). Although the polyculture of sea cucumber with shrimp set a precedent for the reuse of shrimp waste as a food source for sea cucumber, both species in polyculture grew more slowly than those in monoculture, and the ammonium-nitrogen concentration in polyculture system was higher than that in monoculture system, which indicated that polyculture did not improve water quality (Purcell *et al.*, 2006). Moreover, Bell *et al.* (2007) observed that limited growth and mass mortality of sea cucumber occurred when it was reared with shrimp, exhibiting that polyculture was not practicable. In other words, the present polyculture mode makes it difficult to remove shrimp waste by sea cucumber. Thus a new method needs to be found to treat the farming waste from shrimp aquaculture and make it the food source of sea cucumber. In fact, some studies have aimed at the reuse of farming waste from marine animals in sea cucumber aquaculture (Yuan *et al.*, 2006; Slater *et al.*, 2009; Maxwell *et al.*, 2009; Zamora and Jeffs, 2011). However, these studies basically focused on the reuse of farming waste from shellfish and fish such as abalone, scallop, mussel, oyster and salmon. So far there has been no information concerning the use of dried farming waste from shrimp as part of food of sea cucumber.

Spirulina platensis is a blue-green filamentous microalga which is regarded as human health food and animal feed due to its high contents of proteins, vitamins and unsaturated fatty acids. Previous studies indicated that dietary administration of *S. platensis* could increase the phagocytic activity of fish and enhance the resistance of aquatic animals against pathogens (Duncan and Klesius, 1996; Watanuki *et al.*, 2006; Lee *et al.*, 2003; Rahman *et al.*, 2006; Abdel-Tawwab and Ahmad, 2009). However, to our knowledge, there is no published study regarding the use of *S. platensis* for the culture of sea cucumber. In contrast, the macroalga *Sargassum polycystum* and sea mud have been widely used in sea cucumber aquaculture industry (Liu *et al.*, 2009; Slater *et al.*, 2009; Liu *et al.*, 2010; Zhang *et al.*, 2011; Xia *et al.*, 2012).

The goal of this study is to determine whether farming waste from shrimp aquaculture industry can be used for culture of sea cucumber so as to seek a new approach to the reuse of shrimp farming waste.

2 Materials and Methods

2.1 Experimental Diets

Shrimp (*L. vannamei*) waste was collected from eighty shrimp ponds of different shrimp farms in Guangdong Province, China. No fishery drugs were used in the process of shrimp culture. Before shrimp waste was collected, waterwheel aerators were used to aerate shrimp ponds all night to concentrate waste at the outfalls of shrimp ponds spontaneously. On next day, the outfall of the shallow well beside the shrimp ponds was blocked up, and then a part of effluent containing sedimentary waste in the shrimp ponds was discharged into the shallow well. Waste slurry was collected by 0.074 mm spoon net. After the seawater in waste slurry was squeezed out, waste slurry was transferred from the spoon net to a container. These shrimp wastes from different sources were dried by baking at 55℃ and pulverized into ultra-fine powder respectively, and they were mixed well to avoid the possible difference in their nutrition contents.

The initial formulated compound contained 80% powdered algae (53.33% of *Sargassum polycystum* and 26.67% of *Spirulina platensis*) and 20% sea mud (called formulated compound for short). The nutrient in sea mud (% dry weight) included 0.87% ± 0.01% crude protein, 0.32% ± 0.00% crude lipid, 92.62% ± 0.05% ash. *S. platensis* (% dry weight) included 9.19% ± 0.04% crude protein, 0.9% ± 0.01% crude lipid, 39.84% ± 0.03% ash; *S. platensis* (% dry weight) included 61.4% ± 0.03% crude protein, 2.01% ± 0.02% crude lipid, 5.3% ± 0.06% ash.

The final diets used in this experiment were diet A, shrimp waste; diet B, 75% shrimp waste and 25% formulated compound; diet C, 50% shrimp waste and 50% formulated compound; diet D, 25% shrimp waste and 75% formulated compound; and diet E, formulated compound only.

2.2 Experiment Design and Rearing Conditions

Healthy juvenile *S. monotuberculatus* were obtained from Zhanjiang City (Guangdong Province, China). Prior to this experiment, sea cucumber were fed with powdered algae and acclimated for 15 days. After 48 h of starvation, the initial wet body weight of the sea cucumber was measured individually. After that, 120 acclimatized animals with initial wet body weight of (3.97 ± 0.07) g were allocated in equal numbers (n=6) into 20 plastic containers (70 L) to form five groups and four replicates. The five groups were fed with five kinds of experimental diets once a day to excess at about 17:00. Uneaten feeds were siphoned out 24 h later from containers and dried at 65℃ to constant weight in order to determine the ingestion rate. Feces were siphoned out twice a day (at about 9:00 and 16:00) and stored at −20℃ until analysis. Seawater temperature was controlled at (29 ± 2)℃, dissolved oxygen was maintained above 5.0 mg L^{-1} and salinity ranged from 28 to 32. During the experiment, aeration was supplied continuously, and the seawater was exchanged twice a day to ensure the water quality and about one-half volume of the seawater in each container was exchanged each time. The experiment lasted for 70 d. Sea cucumbers were starved for 48 h at the end of the experiment, and then weighed to calculate total specific growth rate.

2.3 Chemical Analysis of Diets and Feces

Diets and feces were analyzed according to AOAC (1990). The acid-insoluble ash (AIA) content of diets and feces was determined with the method of Atkinson *et al.* (1984). The total organic matter (TOM) contents of diets and feces were determined with combustion method recommended by Byers *et al.* (1978) with modifications. The samples were oven dried at 60℃ for 48 h, weighed and then placed in a furnace set at 500℃ for 6 h to ensure complete combustion of organic matter, and then re-weighed. Percentage of TOM was calculated by sample weight lost after combustion.

2.4 Data Processing

The mortality of sea cucumber was recorded during this experiment. Specific growth rate (SGR) and ingestion rate (IR) were calculated according to the equations of Zamora and Jeffs (2012). Food conversion ratio (FCR) was calculated according to a study of Ebrahimi *et al.* (2012). Apparent digestion ratio (ADR) was calculated using the following equations (Deng *et al.*, 2010):

$$SGR\ (\%\ \mathrm{d}^{-1}) = 100\,(\ln W_2 - \ln W_1)/t\,,$$

$$IR\ (\mathrm{g\ ind}^{-1}\ \mathrm{d}^{-1}) = C/n/t\,,$$

$$FCR = C/(W_2 - W_1)\,,$$

$$ADR\ (\%) = 100 - 100 \times \frac{AIA \text{ in diet}}{AIA \text{ in feces}}\,,$$

where, W_1 and W_2 are initial and final total wet body weight of sea cucumber in each container; t is the duration of the experiment (70 d); n is the number of sea cucumbers in each container; and C is the dry weight of food consumed.

2.5 Ammonium-Nitrogen Excretion

Ammonium-nitrogen excretion was determined for one whole day in the middle of this experiment. Different diets supplied for the sea cucumber were put into containers after exchanging the water at 16:30. The concentration of ammonium-nitrogen each container was determined at that time and 24 h later. Ammonium-nitrogen was determined by using the salicylate-hypochlorite method (Bower and Holm-Hansen, 1980). One container without sea cucumber was used as control. Ammonium-nitrogen excretion was calculated using the following equations (Xia *et al.*, 2012, 2013):

$$NH_4\text{-N excretion (mg (g diet)}^{-1}) = (\text{Final } NH_4\text{-N} - \text{Initial } NH_4\text{-N})(\text{mg L}^{-1}) \times \text{Water volume (L)/Feed intake (g)}\,.$$

2.6 Variation of Nutrition Contents in Feces

According to the research of Zamora and Jeffs (2011), the variation of nutrition contents (crude protein, crude lipid and TOM) in the feces of sea cucumbers treated with different diets was compared with that in corresponding diets, and the variation regularity of nutrition content in feces among different diet treatment groups was also analyzed.

2.7 Statistical Analysis

Statistics was performed using software SPSS 11.0 for Windows with possible difference between diets and feces in the same diet treatment group for each nutrient component (crude protein, crude lipid, ash, TOM) being tested by Independent-Samples T Test. Before ANOVA comparisons, data were tested for homogeneity of variance using the Levene's test. Inter-treatment differences of *SGR*, *IR*, *FCR*, *ADR*, ammonium-nitrogen excretion and nutrition contents (crude protein, crude lipid, ash, TOM) in feces were tested by one-way ANOVA. Duncan's multiple range test was used to test the difference among different diet treatment groups. Difference was considered significant when $P<0.05$.

3 Results

3.1 Growth

There was significant difference in *SGR* among differ-

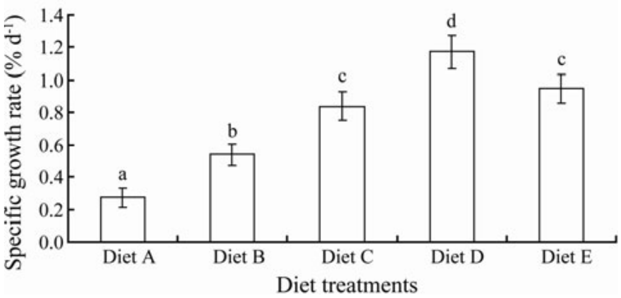

Fig.1 Specific growth rate (*SGR*) of the sea cucumber in this experiment. Different letters indicate significant difference ($P<0.05$), and error bars indicate the standard deviation from mean.

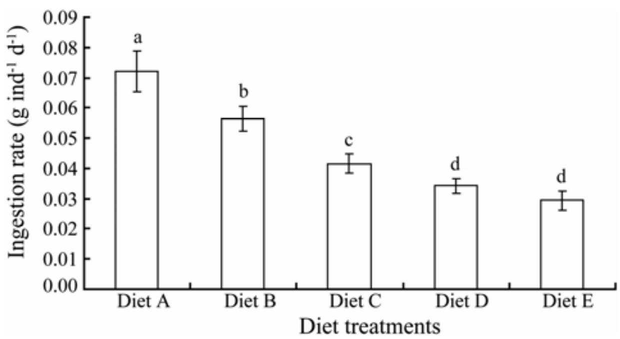

Fig.2 Ingestion rate (*IR*) of sea cucumber in this experiment. Different letters indicate significant difference ($P<0.05$), and error bars indicate the standard deviation from mean.

ent diet treatment groups ($P<0.05$) except for diet C and diet E treatment group ($P>0.05$). The highest and lowest *SGR* occurred in diet D and diet A treatment group, respectively (Fig.1). During this experiment, no mortality occurred in any groups.

3.2 Ingestion Rate

There was significant difference in *IR* among different diet treatment groups ($P<0.05$) except for diet D and diet E treatment group ($P>0.05$). The highest and lowest *IR* occurred in diet A and diet E treatment group, respectively. The average *IR* value decreased gradually as the nutrition content of diets increased (Fig.2).

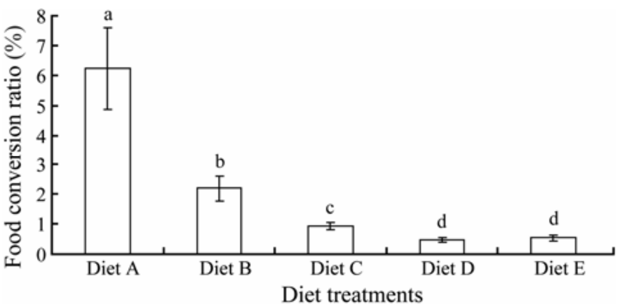

Fig.3 Food conversion ratio (*FCR*) of sea cucumber in this experiment. Different letters indicate significant difference ($P<0.05$), and error bars indicate the standard deviation from mean.

3.3 Food Conversion Ratio

The highest and lowest *FCR* occurred in diet A and diet D treatment group, respectively, and there was significant difference in *FCR* among different diet treatment groups ($P<0.05$) except those between diet D and diet E treatment group ($P>0.05$) (Fig.3).

3.4 Apparent Digestion Ratio

There was significant difference in *ADR* among different diet treatment groups ($P<0.05$) except for diet C and diet D treatment group ($P>0.05$). The highest and lowest *ADR* occurred in diet E and diet A treatment group, respectively. The average *ADR* value increased gradually as the content of shrimp waste in different diets decreased (Fig.4).

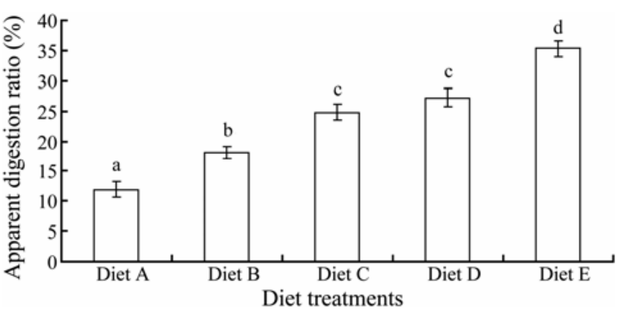

Fig.4 Apparent digestion ratio (*ADR*) of sea cucumber in this experiment. Different letters indicate significant difference ($P<0.05$), and error bars indicate the standard deviation from mean.

3.5 Ammonium-Nitrogen Excretion

There was no significant difference in ammonium-nitrogen excretion between diet B and diet C treatment group, diet C and diet D treatment group, and diet D and diet E treatment group, respectively ($P>0.05$). The highest and lowest ammonia-nitrogen excretion occurred in diet E and diet A treatment group, respectively. The average ammonium-nitrogen excretion value increased gradually as the crude protein content in different diets increased (Fig.5).

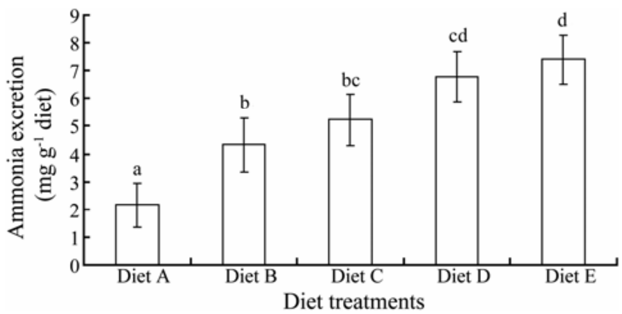

Fig.5 Ammonium-nitrogen excretion of sea cucumber in this experiment. Different letters indicate significant difference ($P<0.05$), and error bars indicate the standard deviation from mean.

3.6 Variation of Nutrition Contents in Feces

The average crude protein content in feces with different diet treatments increased gradually as the average crude protein content in diets increased. There was significant difference in crude protein content among feces with different diet treatments ($P < 0.05$). The average crude protein content in feces from different diets decreased to different extents in comparison with those in corresponding diets, and the difference of average crude protein content between feces and corresponding diets with increased gradually when the average crude protein content in diets increased.

The average crude lipid content in feces from different diets decreased gradually when the average crude lipid content in diets increased. There was significant difference in crude lipid content among feces from different diets ($P<0.05$) except for those between diet A and B, diet B and C, and diet D and E. The average crude lipid content in feces from different diets decreased to different extents in comparison with those in corresponding diets. The difference of average crude lipid content between feces and corresponding diets increased gradually when the average crude lipid content in diets increased.

The average TOM content in feces from different diets increased gradually when the average TOM content in diets increased. There was significant difference in TOM content among feces from different diets ($P<0.05$). The average TOM content in feces from different diets decreased in comparison with those in corresponding diets. Among diets tested, the difference of average TOM content between feces and corresponding diets was similar to each other (Table 1).

Table 1 Nutrient content in diets and feces

Nutrient content		Diet treatment				
		A	B	C	D	E
Crude protein (%)	Diets	13.98±0.09^{A}	15.85±0.11^{A}	17.72±0.04^{A}	19.59±0.12^{A}	21.45±0.09^{A}
	Feces	10.2±0.05^{Ba}	11.09±0.1^{Bb}	11.34±0.13^{Bc}	12.29±0.05^{Bd}	12.54±0.07^{Be}
Crude lipid (%)	Diets	0.65±0.03^{A}	0.76±0.06^{A}	0.87±0.02^{A}	0.97±0.09^{A}	1.08±0.08^{A}
	Feces	0.33±0.05^{Ba}	0.28±0.05^{Bab}	0.25±0.02^{Bb}	0.2±0.03^{Bc}	0.11±0.07^{Bc}
Ash (%)	Diets	45.87±0.12^{A}	44.7±0.09^{A}	43.53±0.08^{A}	42.35±0.02^{A}	41.18±0.04^{A}
	Feces	56.34±0.07^{Ba}	55.44±0.10^{Bb}	54.08±0.06^{Bc}	53.26±0.07^{Bd}	52.28±0.03^{Be}
Total organic matter (TOM) (%)	Diets	54.13±0.12^{A}	55.3±0.09^{A}	56.47±0.08^{A}	57.65±0.02^{A}	58.82±0.04^{A}
	Feces	43.66±0.07^{Ba}	44.56±0.10^{Bb}	45.92±0.06^{Bc}	46.74±0.07^{Bd}	47.72±0.03^{Be}

Notes: Data with different capital letters in the same column are significantly different between diets and feces ($P<0.05$); Data with different small letters in the same line are significant different among the feces from different diets ($P<0.05$).

4 Discussion

4.1 Growth Performance and Apparent Digestion Ratio

In this study, after sea cucumbers ingested pure dried shrimp waste, their total body weight increased, which was not in agreement with the result of Yuan *et al.* (2006) who found that a diet of pure dried bivalve feces led to negative growth of sea cucumber (*A. japonicas*). Zhu *et al.* (2005) reported that the optimum dietary protein and lipid requirements of juvenile sea cucumber were 18%–24% and 5%, respectively. Although the contents of protein and lipid in dried shrimp waste (crude protein 13.98 %, crude lipid 0.65%) used in this experiment were lower than the reported, sea cucumber could compensate for lower nutritional value by increasing the amount of food intake, resulting in weight gain (Xia *et al.*, 2012).

Generally speaking, the growth of animal was affected by different diets through the interaction of *IR*, *FCR* and *ADR* (Yuan *et al.*, 2006). In this study, the *IR* value of diet D was higher than that of diet E while the *ADR* value of the former was lower than that of the latter, and their *FCR* values were similar to each other. Additionally, farming waste of marine animals might provide certain nutrients or digestion regulators to other animals, such as some mineral components which might be vital to the metabolism of sea cucumber (Xu *et al.*, 1999). Maybe the combination of these factors induced the faster growth of sea cucumber fed diet D compared with those fed with diet E.

Different nutrition contents in diets might result in different *IR* values of animals, and a negative relationship was revealed between the *IR* value of sea cucumber and the nutrition contents of different diets (Liu *et al.*, 2009; Yuan *et al.*, 2006). Thus, in this study, it was reasonable that the average *IR* value with different diet treatments decreased gradually as the nutrition contents of different diets increased, which was in accordance with the results in previous literatures (Liu *et al.*, 2010; Xia *et al.*, 2012).

High growth rate was normally accompanied by low *FCR* (Mercer *et al.*, 1993). *FCR* and *SGR* in this experiment mainly reflected this negative relationship except for similar *FCR* values in diet D and diet E treatment group.

In the present study, the average ADR value of sea cucumber decreased gradually as the content of dried shrimp waste in different diets increased, indicating that dried shrimp waste was absorbed hard by sea cucumber. A similar result was also discovered when dried bivalve feces were used in culture of *A. japonicas* (Yuan *et al.*, 2006).

4.2 Ammonium-Nitrogen Excretion

In this study, the average ammonium-nitrogen excretion value of sea cucumber with different diet treatments increased gradually as the crude protein content in different diets increased. Sea cucumber could consume more protein in high-protein diets and less protein in low-protein diets (Table 1), and ammonium-nitrogen excretion could reflect the amount of ingested protein metabolism (Xia *et al.*, 2012), which was a probable explanation for this result.

Maintaining an acceptable water quality is extremely important for sea cucumber. Excessive ammonium in aquaculture water body could be harmful to aquatic animals. In the present study, it seemed that ammonium excretion was not harmful to sea cucumber because of their high survival rate, which suggested that ammonium-nitrogen excretion in this experiment was within a safe range.

4.3 The Variation of Nutrition Content in Feces

In this study, the differences of the nutrition contents (crude protein, crude lipid) between feces and corresponding diets with different diet treatments increased gradually as the nutrition contents (crude protein, crude lipid) in different diets increased, indicating that when the nutrition contents in diets fluctuated within a certain range, the nutrition contents in diets were higher, more nutrients in ingested diets were consumed by sea cucumber. However, the differences of the TOM content between feces and corresponding diets among different diet treatment groups were close to each other, indicating that sea cucumber with different diet treatments had identical ability of consuming organic matter in ingested diets.

It is well known that sea cucumber can consume organic matter from food and convert it into body tissues. During this experiment, no mortality occurred in any groups, meaning that a part of nutrient in ingested diets was used for sustaining their lives. Therefore, the nutrition contents in the excrement might be lower than those in diets, which was a probable explanation for the decrease of the contents of protein, lipid and TOM in feces in comparison with those in corresponding diets. Similar result was reported by Zhou *et al.* (2006) that a small reduction of organic content in scallop lantern net waste was found when they were reared with *A. japonicus*. In other words, sea cucumber had the capability of reducing organic pollution of shrimp farming waste.

5 Conclusions

In this study, after sea cucumber ingested diets containing different quantity of dried shrimp waste, their total body weight increased without mortality. Hence, it was feasible that dried shrimp waste could be applied to sea cucumber aquaculture industry. This study showed that sea cucumber exhibited the fastest growth when they were fed with the diet containing 25% dried shrimp waste and 75% formulated compound (20% sea mud and 80% powdered algae), indicating that dried shrimp waste could become a component in the formulation of sea cucumber feeds. Moreover, this work may provide another approach to reusing the waste from shrimp aquaculture industry.

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