Application of dried shrimp head in the diets of sea cucumber *Holothuria scabra*

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Abstract

This study aims to determine the suitability of dried shrimp head as part of the ingredient in formulated diet to promote growth of sea cucumber in captivity. The sea cucumber *Holothuria scabra* were divided into two tanks to test two different diets: Diet A (commercial diet supplemented with dried shrimp waste) and Diet B (commercial diet without dried shrimp waste). A total of 20 pieces of *H. scabra* per tank for each diet were involved in the experiment that lasted for 8 weeks. The weight increment of *H. scabra* from each tank was recorded weekly and survival rate (SR), weight gain rate (WGR) and specific growth rate (SGR) was calculated. Overall, *H. scabra* fed with Diet A showed higher WGR than *H. scabra* fed with Diet B, with increment of 30.57 % (12.10 g) recorded in Diet A to 14.26 % (11.18 g) for Diet B from their average initial weight. The overall SGR of Diet A and Diet B were 0.48 % and 0.24 %, respectively. SR remaining at 100 % for all treatments. Statistical analysis showed the average wet weight of Diet A was significantly higher (p<0.05) than Diet B. Results showed the ability of sea cucumber *H. scabra* to consume and digest diet containing dried shrimp head, suggested that there is potential in using dried shrimp head to promote growth rates of sea cucumber. Using dried shrimp heads can be considered to make good use of shrimp farming waste.

Keywords: Dried shrimp head, *Holothuria scabra*, Sea cucumber diets

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1-Marine Ecology Research Centre (MERC) is located at Gaya Island, Kota Kinabalu, Malaysia. MERC is privately funded and dedicated to marine conservation since 2007. MERC actively propagates and release marine organisms for restocking in depleted habitats.
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Introduction

Sea cucumbers are a high-value seafood product that being harvested worldwide for their body wall (bêche-de-mer). The increasing demand of sea cucumber has led to over exploitation throughout the world (Hamel *et al.*, 2001; Conand, 2004; Lovatelli *et al.*, 2004; Uthicke, 2004) and has caused a significant decline in natural populations in almost all countries that harvest them (Conand, 2004). This has encouraged the development of sea cucumber aquaculture. However, knowledge of dietary requirements has limited the potential expansion of this activity. Limited data regarding the feeding behavior, nutrition requirements and artificial feeds for this species is available (Chen *et al.*, 2015). There are also no specific formulated diets that meet the nutritional requirements of these organisms under intensive farming (Zacarias-Soto and Olvera-Nova, 2015).

Sea cucumbers farmed in captivity are mostly co-cultured in shrimp ponds, feeding on the shrimp waste and excess food (Pitt *et al.*, 2004), or cultured in abandoned shrimp ponds using the remaining natural food resources, with no supply of formulated food (Agudo, 2006). For sea cucumbers farmed in tanks, they are fed with formulated diets. Plant-based ingredients such as soy or corn meals and other grains or distillery by-products have been included in diets for sea cucumber farming (Yuan *et al.*, 2006; Maxwell *et al.*, 2009; Seo *et al.*, 2011). Several species of kelp have also been grounded into paste and frozen or dried and placed in broodstock tanks (Ito, 1995; Yanagisawa, 1995). Battaglene and Bell (1997) stated that *Holothuria scabra* broodstock in India and Indonesia were fed soybean powder, rice bran, chicken manure, and ground algae and prawn-head waste. Agudo (2006) and James (1996) also reported on using shrimp head waste as part of the ingredient in the formulated feed for sea cucumber's broodstock conditioning in captivity.

Dried shrimp waste is the undecomposed dried waste of shrimp (Heuzé and Tran, 2015). It can contain whole shrimps and/or shrimp parts such as the heads, appendages and exoskeleton (Fanimo *et al.*, 1996; Fanimo *et al.*, 2000). They can be grounded down and the powder used as an animal feed supplement, bait or fertilizer, as well as in chitin production (Yan and Chen, 2015). Shrimp production represented 15% of all globally traded fishery product in 2013, and Asia contributed 85% of shrimp aquaculture production and 74% of wild shrimp captured (FAO, 2014; Mirzah *et al.*, 2020). However, approximately 70% of total shrimp landings turned into waste (Heuzé and Tran, 2015), due to most shrimps are sold headless (Hossain *et al.*, 2018). Moreover, the increase in shrimp farming industry has led to the more production of shrimp waste (Fanimo *et al.*, 2000). The increased quantity of shrimp waste has raised environmental problems and is a waste of natural resources (Mirzah *et al.*, 2020). Thus, using shrimp waste as feed is considered a part of waste management in the shrimp industry.

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Shrimp head has rich source of protein, chitin, carotenoids, enzymes and other nutritive components (Yanar *et al*., 2004; Holanda and Netto, 2006; Samar *et al*., 2013, Thongprajukeaw, 2014), which can enhance gonad development of cultured animals (Akiyama *et al*., 1991; Gowsalya and Kumar, 2018). It also has higher mineral and calcium content than fish meal, as well as some amino acids such as aspartic acid, glutamic acid, leucine, lysine and argentine (Hossain *et al*., 2018). Shrimp head is considered a good ingredient for fish because of its high crude protein content and digestibility (Göhl, 1982; Heuzé and Tran, 2015), stimulating fish growth (Hertrampf *et al*., 2000; Heuzé and Tran, 2015) and used by fish nutritionists in diets to produce desired coloration in trout and salmon (Rosenfeld *et al*., 1997).

The objective of this study is to determine the suitability of dried shrimp head as part of the ingredient in formulated diet to promote growth of sea cucumber in captivity. Captive animals tend to lose their body mass over the period of captivity. Most studies suggested that density of sea cucumber in tank and lack of appropriate diets are the main causes for the poor performance of sea cucumber held in captivity (Turner, 2015). If proper food is not provided, the animals shrunk and the gonad is re-absorbed, rendering the material unfit for spawning purposes. Thus, feeding them nutritional feed is an important aspect to improve the growth performance of sea cucumber broodstock. In addition, good quality feed given to broodstock will also promotes better reproduction process (Nzohabonayo *et al*., 2017). Using dried shrimp heads can be considered to make good use of shrimp farming waste.

Materials and methods

Experimental design and rearing conditions

The experiment was carried out in Marine Ecology Research Centre (MERC), located in Malohom Bay of Gaya Island, Sabah. The *Holothuria scabra* collected from Kudat and Gaya Island was divided into two tanks: Tank A and Tank B, to test two different diets: Diet A (commercial diet supplemented with dried shrimp waste) and Diet B (commercial diet without dried shrimp waste).

An open flow through system was used with 1.085 m^3 (1.445 x 1.615 x 0.465 meter) concrete tanks, where each tanks was filled with continuously running seawater pumped directly from the sea and through a sand filter. The system also included continuously supply of aeration. This system helps to ensure and reduce fluctuation of the water quality parameters in the tanks. Sand collected from seagrass area (grain size 35-1000 micron) was used as substrate in tanks, and was changed every 2-3 weeks to avoid growth of bacteria that would cause lesion to the *H. scabra* in the tanks. The tanks were situated under open environment, with natural sunlight during day time (photo period approximately 8-10 hours). By designing the tank environment as close to the nature

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environment also reduced stress on *H. scabra* that being kept in confined space.

Experimental diets

Ingredients and composition of two experimental diets: Diet A (with dried shrimp head) and Diet B (without dried shrimp head) as shown in Table 1. Unwanted head parts of giant tiger prawn or Asian tiger shrimp (*Penaeus monodon)* were collected from Bayu Aquaculture Sdn. Bhd. marine fish and shrimp farm in Tuaran, Sabah. No chemicals were used in the entire process of shrimp culture. Shrimp heads collected were then dried under the sun and stored in a cool, dry place.

Table 1: Ingredients and composition of two experimental diets.

Ingredients	Diet A	Diet B
Dried shrimp head	25 %	0 %
Soybean meal	23 %	35.5 %
Shrimp feed	23 %	35.5 %
Squid oil	2.5 %	2.5 %
Soybean oil	2.5 %	2.5 %
Spirulina powder	8 %	8 %
Vitamin premix	4 %	4 %
Mineral premix	4 %	4 %
Agar	4 %	4 %
Water	3 %	3 %

Prior to the preparation of diet, shrimp heads were grounded before mixing and cooking with other ingredients. The composition of dried shrimp head in Diet B was replaced with soybean meal and *Litopenaeus vannamei* shrimp feed, as both ingredients were also part of protein supply in the diet. Squid oil and soybean oil were served as the lipid sources while spirulina powder was the carbohydrate source in the experimental diets. Agar was used to bind all the mix ingredients for easy storing and feeding.

The first step in preparation of the feed was to add agar into boiled water and then left aside to reduce the temperature of the liquid agar. Other ingredients were then added and mixed evenly before allowing the agar to cool and turned into solid stage. The mixture was then transferred into a pan for further cooling. This would be stored inside a container and kept refrigerated.

Feeding trials

The number of *H. scabra* involved in the experiment was 20 pieces per tank for each diet. Initial weight recorded was 39.58±3.31 g for Tank A (Diet A) and 78.41±6.67 g for Tank B (Diet B). During the experiment period that lasted for 2 months (8 weeks / 56 days), the sea cucumber in both tanks was fed once per day in the evening, at about 1630 hour. Amount of feed given were based on 3% of their total wet weight recorded weekly. Uneaten feed on the following day were mixed with the sand substrate in tanks so that *H. scabra* can continue feeding on the diet when grazing on the sand.

Data recording

The weight of *H. scabra* were measured at the beginning of the experiment and continue to be measured weekly to record their growth in terms of wet weight (g). The process included removing the *H. scabra* out from the tanks, waited a few seconds for them to expel the water, weighing them, and returning them to their tank. Survival (%) was measured by

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taking a weekly record of the living organisms.

Data calculation

The weight increment of *H. scabra* from each tank was recorded weekly. Survial rate (SR), weight gain rate (WGR) and specific growth rate (SGR) were calculated as below following equation of Zamora and Jeffs (2012), and Wang *et al.* (2016):

SR (%) = (N_2 / N_1) x 100

WGR (%) = [(W_2 - W_1) / W_1] x 100

SGR (%) = [(ln W_2 - In W_1) / T] x 100

where N_1 is the number of individuals alive at the beginning of experiment and N_2 is the number of individuals surviving at end of the experiment; W_1 and W_2 are initial and final average wet weight recorded. T is the experimental period in days.

Statistical analysis

Statistical analysis was performed by the software SPSS with possible differences between the two diet treatments being tested by using independent-samples t-test. Differences were considered significant at a probability level of less than 0.05 (p<0.05).

Results

Summary of weekly weight measurement of *H. scabra* fed with two different diets is shown in Table 2. Effects of different diets on weight gain rate (WGR) and specific growth rate (SGR) of *H. scabra* is shown in Table 3.

Overall, *H. scabra* fed with Diet A (commercial diet with dried shrimp head) showed significant higher weight gain compared to *H. scabra* fed with Diet B (commercial diet without dried shrimp head) after the 8 weeks of trial feeding, with increment of 12.10 g (30.57 %) and 11.18 g (14.26 %) from their average initial weight, respectively.

Table 2: Weekly weight (g) measurement record of *Holothuria scabra* fed with different diets.

Week	Weight (g)	
	Diet A	Diet B
0 (Initial)	39.58 ± 3.31	78.41 ± 6.67
1	44.89 ± 3.88	90.54 ± 9.48
2	41.91 ± 3.43	88.67 ± 9.78
3	37.35 ± 2.14	104.80 ± 10.64
4	38.74 ± 2.64	97.80 ± 9.87
5	44.70 ± 2.96	106.98 ± 10.38
6	47.14 ± 2.97	108.32 ± 9.42
7	42.00 ± 3.00	85.69 ± 9.35
8	51.68 ± 4.40	89.59 ± 10.59

Both diets showed almost similar trend throughout the 8 weeks, except in week 3 and 4, where *H. scabra* fed with Diet A showed decrement in their average weight in week 3 and increment in week 4, and vice versa in Diet B. The decrement in average weight of *H. scabra* could be attributed to some *H. scabra* developing lesion during the treatment period. The SGR was of the same order as that of WGR. The overall SGR of Diet A and Diet B after 8 weeks (56 days) of treatment were 0.48 % and 0.24 %, respectively. The highest WGR and SGR were observed in week 8 and week 3 in Diet A and Diet B, respectively, whereas the lowest WGR and SGR was observed in week 7 for both diets.

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Statistical analysis showed there was a significant difference between the average wet weight, where Diet A was significantly higher (p<0.05) than Diet B. No mortality was observed for both diets during the experiment period, with survival remaining at 100% for all treatments.

Table 3: Effects from different diets on WGR and SGR of *Holothuria scabra*.

Growth Performance	Diet A	Diet B
Week 1 (1 - 7 day)		
Weight gain (g)	5.32	12.13
WGR (%)	11.83	15.47
SGR (%)	1.80	2.05
Week 2 (8 - 14 day)		
Weight gain (g)	-2.98	-1.87
WGR (%)	-6.64	-2.06
SGR (%)	-0.98	-0.30
Week 3 (15 - 21 day)		
Weight gain (g)	-4.56	16.13
WGR (%)	-10.88	18.19
SGR (%)	-1.64	2.38
Week 4 (22 - 28 day)		
Weight gain (g)	1.39	-7.0
WGR (%)	3.72	-6.68
SGR (%)	0.52	-0.99
Week 5 (29 - 35 day)		
Weight gain (g)	5.96	9.18
WGR (%)	15.38	9.38
SGR (%)	2.04	1.28
Week 6 (36 - 42 day)		
Weight gain (g)	2.44	1.34
WGR (%)	5.46	1.25
SGR (%)	0.75	0.18
Week 7 (43 - 49 day)		
Weight gain (g)	-5.14	-22.63
WGR (%)	-10.90	-20.89
SGR (%)	-1.65	-3.35
Week 8 (50 - 56 day)		
Weight gain (g)	9.68	3.9
WGR (%)	23.05	4.55
SGR (%)	2.96	0.64
Overall weight gain (g)	**12.1**	**11.18**
Overall WGR (%)	**30.57**	**14.31**
Overall SGR (%)	**0.48**	**0.24**
Survival rate, SR (%)	**100**	**100**

Discussion

The results from this study showed that sea cucumber (*H. scabra*) was able to consume and digest diet containing dried shrimp head. Throughout the experiment period, no mortality was recorded. However, some *H. scabra* in both treatment had developed lesion during the experiment and caused them shrinking in size and thus fluctuation in their weekly average weight. Decrease in weight of *H. scabra* was observed in week 2 and 7 for both diets. Weight decrements were also observed in week 3 for Diet A and week 4 for Diet B. Lesion was spotted on one to three individuals while others not displaying lesion were also observed to have shrunk in size. The reason could be bacteria found in sand substrate collected from wild and also parasites such as pea crab that were introduced accidentally from the sand. Nevertheless, the body weight of *H. scabra* fed with Diet A was able to compensate and increase more than those that fed with Diet B after recovery. This suggested that part of the nutrient in the dried shrimp head was used to maintain and promote the growth.

Sand was used during the experiment to maintain and promote the *H. scabra* growth in captivity. This was agreed by Robinson *et al.* (2013), Shi *et al.* (2013), and Qiu *et al.* (2014), which stated that keeping sea cucumber in tanks with sand actually promotes growth, weight gain, and increases the SGR. However, sand collected from wild tends to mix with many microbes such as bacteria, fungi, protozoa and virus, as well as parasitic organisms such as

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gastropods, flatworms and crustacean. The invasive organisms that usually cause lesion in *H. scabra* have been reported as bacteria, fungi, and protozoa. (Deng *et al*., 2009; Lavitra *et al.*, 2009). In this study, freshly collected sand was sieved and unwanted organisms were removed. The sand in tank was changed at least once every two weeks to reduce the growth of harmful bacteria.

Final weight measurement of *H. scabra* fed with Diet A showed significantly higher increment compared to Diet B, which might be related to the higher crude protein content in dried shrimp head. This is supported by Nargis *et al.* (2006), which reported the shrimp head waste contained 49.47 % of crude protein. Other than that, Nwanna (2003) reported the shrimp head silage meal contain crude protein of 58.96 %. These reports showed that meal contain shrimp head is rich in protein and compares favorably with other conventional dietary protein sources, such as fish meal at 40 to 70 % depend on raw materials quality (Masagounder *et al*. 2016), and soybean meal at 43 to 53 % (Heuzé *et al*, 2020).

In this study, the composition of dried shrimp head in Diet B was replaced by soybean meal and shrimp feed. The major components of protein in shrimp feed used in this study were wheat flour, soybean meal and fish meal, which consist of 40% crude protein. Besides, soybean products are relatively high in carbohydrate, low crude fat and crude protein levels compared to those found in fish meal (Heuzé *et al*., 2020). This explained the final weight increment, where *H. scabra* fed with Diet A showed significant higher weight gain compared to *H. scabra* fed with Diet B. The result is supported by Krishnan (1968), stating that carbohydrates appear to be less important than protein and lipid for sea cucumber gonad development. Similarly, in the study by Seo and Lee (2011), sea cucumber juveniles show stronger growth when fed diets rich in protein. Moreover, shrimp head was also found to be rich in mineral content especially calcium, phosphorus, sodium and zinc (Ibrahim *et al*., 1999). In term of cost benefit, shrimp head waste is considered a low cost processing product compare to other commercial feed. It only need to be dried under the sun or oven and grounded into powder form before used as feed. The cost of production on a commercial scale is expected to be minimized.

So far no studies have been conducted to estimate the effect of diet containing shrimp head on the growth of sea cucumber. Used of shrimp waste product as part of the ingredients in the sea cucumber diets are mostly in the form of fresh wet waste which consist of feces and residual feed of shrimp. James (1996) mentioned that the sea cucumbers accepted well on feed with 6.5 % protein which was prepared with shrimp head waste, soybean powder and rice bran. However, no record or statistical analysis has been done to estimate the growth rate. Study by Chen *et al.* (2015) reported that the sea cucumber fed with 50 % wet shrimp waste achieved higher SGR (0.51) compare to diet contain only the commercial powdered alga after 60 days

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of experimental period. The results were relatively higher than the SGR (0.48) obtained in this study, which might be cause by the fluctuation of weekly SGR due to lesion on the *H. scabra*. Besides, Slater *et al.* (2009) reported that sea cucumber fed with mussel waste diet exhibited an average SGR of 0.32 over 3 months, comparatively lower than present study, showing the potential of dried shrimp head to be used as part of the ingredient in sea cucumber diets

Conclusions

Results from the present study suggested that there is potential in using dried shrimp head to promote growth rates of sea cucumber. Formulated diet included dried shrimp head at 25% level can improve the performance of sea cucumber in terms of growth in 8 weeks of experiment when compared to diet without dried shrimp head included. The use of dried shrimp head in sea cucumber aquaculture can also reduce the pollution waste from shrimp aquaculture industry. However, future research is needed to determine the optimum dried shrimp head composition in the diet that could promote maximum growth and ingestion rate, and the effects of shrimp head waste on sea cucumber gonad development. The type of substrate or sediment used in the tank also needs to be examined to prevent injury on sea cucumber that could affect the growth performance.

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