Assessment on dietary protein requirement of sandfish (Holothuria scabra) through growth response and plasma insulin-like growth factor-1 profile

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Key words: Protein doses, specific growth rate, IGF-1.

http://dx.doi.org/10.12692/ijb/5.11.86-91 Article published on December 08, 2014

Abstract

A growth trial was undertaken to investigate dietary protein requirement of the sub adult sandfish, Holothuria scabra, using correlation of growth rate and plasma IGF-1 concentrations. The sand fish (n = 6 per treatment, average wet weight 21.5g ± 2.5 S.D.) were fed one of the seven diets containing various levels of proteins at the ration of 3% of the body weight, once a day for 6 weeks. The analysis of IGF-1 was performed from pooled data per treatment using Bovine IGF-1 kit. Results revealed that protein doses affected the specific growth rate (SGR), and the food conversion ratio (FCR) with the 10% protein dose gave the best result. Plasma concentration of IGF-1 was positively correlated with protein dose and specific growth rate in the sandfish. The 10% protein dose produced highest SGR, but the IGF-1 concentration was not significantly different from other treatments. It could be concluded that both SGR and plasma IGF-1 concentration were affected by protein dose in the diet. The sandfish was expected to require 10-35% protein in the diet as suitable rearing dose.

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Introduction
Sandfish (*Holothuria scabra*) is one of the important holothurian species in Asian market. It is known to have various beneficial bioactive content as medicinal and cosmetic raw materials. It is also a popular culinary due to its high protein and low fat content (Chen, 2004; Choo, 2004). Sandfish culture in Indonesia has not been developed as other cultivated marine organisms, such as fish or shrimp, but it is technically and economically possible (Giraspy and Walsalam, 2010). One of the problems in the sandfish culture is no formulated diet available to support the growth.

Growth is highly affected by environmental factors and nutritional status and internal regulations of the growth hormones. IGF-1 is one of the hormones involved in growth regulation of several vertebrates. Several studies found some correlation between IGF-1 plasma and growth. In many species, growth is indicated by the increment of IGF-1 content. It is found that fish with high growth rate have higher plasma IGF-1 concentration than the slow growth rate fish (Dyer *et al.*, 2004; Li *et al.*, 2006; Bower *et al.*, 2008).

IGF-1 circulation is manipulated by food availability in which increase in IGF-1 concentration occurs with increased protein level. Change in the nutritional status producing change in growth rate can be identified through alteration of IGF-1 plasmic level. Perez-Sanchez and LeBail (1999) suggested the use of IGF-1 as a marker for growth performance and nutritional status of the cultivated organisms.

It is still unclear the proper protein doses in the diet needed to support the growth of the sand fish. Some studies have investigated the dietary protein content for the sandfish growth by providing additional food, such as shrimp head wastes (James, 1996), seaweed meal, *Undaria pinnatifida*, (Ito and Kitamura, 1998), mixture of shrimp pellet and wheat flour (50% protein), chicks feed (18% protein), mixture of *Gracillaria verrucosa* and wheat flour, mixture of seagrass leaves and wheat flour. The findings showed only small difference in growth rate (Pitt *et al.*, 2001). This feeding trial aimed to evaluate the dietary protein content to support the growth of the sand fish by using IGF-1 as growth indicator as well as to evaluate the feed efficiency. The study used different protein content from 5-35% based on the proximate analysis of the sub adult sandfish body wall as well as dietary protein content of other holothurians species.

Materials and method
Experimental Diet Formulation
Seven test diets were made isoenergy (~3.6 kcal/g) with protein content of 5%, 10%, 15%, 20%, 25%, 30% and 35%, respectively. The test diet formulation and its proximate composition were presented in Table 1. Fish meal (59.7% CP) was protein source of the test feed. Fat and carbohydrate were obtained from fish oil and tapioca starch. The diet was added with mineral mix and vitamin mix to meet the mineral and protein requirements. It was made in dry pellet form and stored in a refrigerator at -20°C up to use.

Research design
The sandfish were collected from Central Moluccas, the province of Moluccas. They were placed in seawater-containing aquarium with no substrate and starved for 24 hours before weighed and randomly distributed in other 40x70x40 cm culture aquaria containing fine sand-coral pieces substrate. The aquaria were aerated and supplied with filtered seawater and facilitated with running water system. Substrate replacement was done once a week. Water temperature and salinity were daily monitored and maintained at 26-31°C and 31-32 ppt respectively.

Fourty-eight individuals of average fresh weight of 21.5 g ± 2.5 were randomly distributed in 24 aquaria, each of which was given one of 7 test feed and control without feeding. Each treatment had 3 replications. Feeding was done once a day 3% (Hartati *et al.*, 2005) of the body weight at 16.00 O’clock. Before feeding, water input flow was closed and water level reduced up to about 5 cm of the surface substrate to mimic the tidal condition in nature. Aeration was also stopped
for a while that the feed could disperse and reach the substrate surface. The water level was relifted after 4-6 hours later.

The sandfish weight was recorded once in two weeks to adapt the number of feed. At the end of culture period, all sandfish were taken their blood. Then they were placed into the no substrate-aquaria and starved for 24 hours before weighing their final weight. The specific growth rate (SGR) was determined as:

\[ \text{SGR, \%body weight/day} = \frac{(\ln Wt - \ln Wo)}{t} \times 100 \]

\( Wt = \) fresh weight at the end of experiment,
\( Wo = \) fresh weight at the initial experiment and \( t = \) time (42 days)

**Plasma sampling and IGF-1 concentration measurement.**

The sandfish coelomic fluid was collected withdrawn using a 1 cc heparinized syringe from lateral side of the body (Xing et al., 2008) and put into Eppendorf tube and centrifuged at 6000 x g for 10 minutes. The supernatant was taken using a micropipet and moved into new Eppendorf, labelled and stored at -20ºC up to further analysis. The plasma IGF-1 content was measured following ELISA kit protocol (BT Laboratory Cat. No. E0017Fi).

**Data Analysis**

Data were presented as mean ± SD. Each value was mean of 3 replications. One way ANOVA was used to compare mean of treatment and then tested with least square difference (Fischer LSD). The treatments were significantly different if P<0.05.

**Results and discussion**

Sandfish juveniles fed on different protein level feed for 6 weeks indicated varied specific growth rate (SGR) between -0.4 in unfed group-1.4%bw/day in 10% protein group. The highest SGR was recorded in the group of 10% protein feed. Increase in dietary protein level up to 35% did not give statistically different growth response from that of 10% protein level (Fig. 1). Feed of 5% protein level seems not enough to push the sandfish growth since the SGR obtained is lower than that using higher protein content feed. There was also no significant difference in the SGR among feed protein of 15-30% (Fig. 1).

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Dietary Protein (%)</th>
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<tr>
<td></td>
<td>5%</td>
</tr>
<tr>
<td>Fish meal</td>
<td>7.80</td>
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<tr>
<td>Fish oil</td>
<td>10.00</td>
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<tr>
<td>Tapioca starch</td>
<td>60.40</td>
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<tr>
<td>Vitamin mix</td>
<td>5.00</td>
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<tr>
<td>Mineral mix</td>
<td>5.00</td>
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<tr>
<td>Binder</td>
<td>11.80</td>
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<tr>
<td>Total</td>
<td>100</td>
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<table>
<thead>
<tr>
<th>Proximate composition (%)</th>
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<tbody>
<tr>
<td>Dry matter</td>
</tr>
<tr>
<td>Crude protein</td>
</tr>
<tr>
<td>Crude fat</td>
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<tr>
<td>Ash</td>
</tr>
<tr>
<td>Extract without nitrogen</td>
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<td>Energy (kkal/100 g)</td>
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The SGR obtained in this study is lower than that reported in Hartati et al. (2005) and Pitt et al. (2001). Moreover, Purcell and Simutoga (2008) and Tsiresy et al. (2011) found that the growth rate of the sandfish was slow enough ranging from 0.04 to 0.23 g/day in spite of living in rich nutrient-waters. Tsiresy et al.(2011) also reported that sandfish growth rate could reach 1.8 g/day. There are differences between the present study and the previously published studies so that the present results could not directly compared to previous findings, such as growth measurement unit or sandfish size used. Yanagisawa (1998) suggested that different sandfish size had different growth rate.
The present and other previous studies have shown varied growth rate of the sandfish. Hartati et al., (2005) and the present study show that good growth occurs in low protein content feeding. Similar findings were also recorded in other sandfish species, such as *Apostichopus japonicus* (Yuan et al., 2006; Liu et al., 2009). Yuan et al. (2006) claimed that in nature, sandfish generally fed on low nutritive value food in high number. On the other hand, in high availability of good quality food, the sandfish tend to reduce number of food consumed. Tsiresy et al. (2011) supposed that it was dealt with the sandfish disability to maximally utilize the available nutrition. This disability is indicated by high food conversion ratio ranging from 2 to 8.8. The high FCR reflects high nutrient content unused optimally or released in feces, and only small part is deposited for growth (NRC, 1993). Low energy deposited for growth was also reported in other sandfish, *Apostichopus japonicus*. Liu et al. (2009), in their budget energy study of the sandfish found that the energy deposited for sandfish, *Apostichopus japonicas*, growth was much lower than that lost through feces or respiration. This study also agrees with many other reports on vertebrates including fish, for instance, Perez-Sanchez et al. (1995) and Qiang et al. (2012), showing positive correlation of IGF-1 plasma level with dietary protein content or SGR. Circulation of plasma IGF-1 in the sandfish was shown to be affected by dietary protein content. There is an indication that increasing protein content will produce more sandfish plasma IGF-1.

The plasma IGF-1 concentration is closely related with growth. In our knowledge, this study is the first in measuring the IGF-1 content plasma of the sandfish and its correlation with the protein dose or SGR. There is no previous data on the IGF-1 level of the sandfish in nature or aquaculture despite the study limited to small sample size.

The present study revealed that IGF-1 plasma concentration was significantly affected by nutritional status, in this case feed protein dose and SGR as well (Fig. 2 and 3). This study also agrees with many other reports on vertebrates including fish, for instance, Perez-Sanchez et al. (1995) and Qiang et al. (2012), showing positive correlation of IGF-1 plasma level with dietary protein content or SGR. Circulation of plasma IGF-1 in the sandfish was shown to be affected by dietary protein content. There is an indication that increasing protein content will produce more sandfish plasma IGF-1.

It is interesting in this study that no significant difference was recorded between the plasma level of IGF-1 of the unfed sandfish and that of those fed with 30% protein. Other interesting evidence was that the sandfish group fed on 10% protein and possessing the highest SGR had sufficiently low IGF-1 content and no difference from other test sandfish of low SGR (Fig. 3). It is still unknown whether it resulted from other factor like stress or unfavorable environmental condition. However, it, of course, needs further evidences. Based on our observations, the sandfish growth under 10% protein feeding had growth deceleration in the last two weeks. Dyer et al. (2004) found that IGF-1 plasma concentration is a good
indicator of final growth performance measured at the short time intervals. It is assumed that growth deceleration occurring in feeding of 10% protein caused low concentration of IGF-1 plasma in the sandfish group. It proved that the IGF-1 concentration is highly sensitive to the nutritional status and could become growth indicator of the sandfish.

Conclusion
This study exhibited that IGF-1 plasma concentration was sensitive to the sandfish nutritional status. Based on this study, we are in agreement that the IGF-1 content is potential as growth rate indicator and tool for evaluating the feed performance. Based on SGR and IGF-1 content evaluation, it could be concluded that the sandfish requires protein range of 10-35% in the diet with 10% protein-dose give the best result in support young sandfish growth.

Acknowledgment
Great appreciation is given to Mariculture Office, Ambon and Biochemical Laboratory of FMIPA, Brawijaya University, Malang, that supported this study. We also thank to the Directorate General of Higher Education for financial support of this study through Doctoral Research Grant 2013/2014.

References


institution 46, 66-70.


