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GONAD DEVELOPMENT OF SANDFISH (*Holothuria scabra*) FED WITH VITAMIN E-SUPPLEMENTED DIET

Sari Budi Moria Sembiring[#] and Jhon Harianto Hutapea

¹Institute for Mariculture Research and Fisheries Extension
Jl. Br. Gondol Ds. Penyabangan Kec. Gerokgak Kab. Buleleng, Kotak Pos 140, Singaraja 81101, Bali, Indonesia

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ABSTRACT

The experiment was conducted to evaluate the effect of vitamin E on the gonad development of sandfish, *Holothuria scabra*. Four experimental diets were formulated to contain different levels of vitamin E, i.e. 0, 150, 300, and 450 mg/kg diet. Dry diets were prepared at the same protein level (14%). The experiment was arranged in a completely randomized design with four treatments and three replicates. Before the experiment started, the internal organs of broodstock were removed (evisceration) by injecting KCl 1 N reagent into their body, and the gonads were observed to determine the sex of each broodstock. Mean total length and body weight of broodstock after the internal organ removal were 10.95 ± 0.37 cm and 61.12 ± 2.28 g, respectively. Three broodstock (one male and two females) were allocated into each plastic bucket with a volume of 150 L. Each plastic bucket was equipped with aeration and flow-through water system. Broodstock fed with experimental diet once daily at 3% of total biomass for 90 days. The results showed that vitamin E has significant effects on percentage of gonad maturation stages and gonad-somatic index (GSI) ($P < 0.05$). Vitamin E at 300 mg/kg diet was the best for gonad maturation of sandfish broodstock. Furthermore, 55.5% and 11.1% of broodstock fed a diet containing 300 mg vitamin E/kg diet reached gonad maturity stage-III and stage-IV, respectively, with GSI of 12.21%.

KEYWORDS: gonad maturation; sandfish; *Holothuria scabra*; vitamin E

INTRODUCTION

Holothuria scabra Jaeger, known as sandfish is one of the most valuable sea cucumber species in the Asian market since 1980 (Junus *et al.*, 2018). Its popularity has led to over-exploitation of its wild stock and currently listed in the International Union for Conservation of Nature (IUCN) as nearly endangered species (Purcell *et al.*, 2014). India and Papua New Guinea have issued the regulation to prohibit trading of sea cucumber (Purcell *et al.*, 2014; Purdy *et al.*, 2017) but since 2017 sea cucumber fishery is reported to be reopened (Hair *et al.*, 2018). There is potential for sea cucumber breeding, culture, and restocking to assist in sustainably maintaining wild populations (Giraspy & Walsalam, 2010).

To develop sea cucumber culture, the major problem is the availability of seeds. Improving

broodstock quality by feeding them with very good feed is one of the possibilities to increase both the number and quality of seeds (Aryani & Suharman, 2015). According to Nzohabonayo *et al.* (2017), a good quality feed given to broodstock will promote good reproduction process. Cultured sandfish broodstock needs longer time to mature and re-mature after spawning (Sembiring *et al.*, 2017). Our proposed alternative to improve the reproduction performance of sandfish broodstock is by feeding them with vitamin E-supplemented feed which has shown to be effective for other freshwater fish and invertebrate species such as Mali fish (*Labeobarbus festivus*) need 150-450 mg/kg feed (Aryani *et al.*, 2014), goldfish (*Carassius auratus*) need 375 mg/kg feed (Arfah *et al.*, 2013), freshwater lobster, *Astacus leptodactylus* need 1,000 mg/kg feed (Barim-oz *et al.*, 2011). Guppy fish, *Poecilia reticulata* need vitamin E 1,000 mg/kg feed for growth and good reproduction performance (Mehrad & Sudagar, 2010). On the other hand, information about the optimum level of vitamin E for sea cucumber is still very limited. Vitamin E requirement to improve growth of juveniles for *Sticophus japonicus* is 23.1-44.0 mg/kg feed (Ko *et al.*, 2009).

[#] Correspondence: Institute for Mariculture Research and Fisheries Extension. Jl. Br. Gondol, Kec. Gerokgak Kab. Buleleng, Kotak Pos 140, Singaraja 81101, Bali, Indonesia
Phone: + 62 362 92278
E-mail: moriasembiring@yahoo.co.id

Vitamin E is soluble in fat and contains four natural tocopherols and tocotrienol. Among four forms of tocopherol, α -tocopherol has the highest activity of vitamin E (NRC, 1993). α -tocopherol also has a significant role in improving broodstock reproduction due to its function as antioxidant and able to maintain fatty acid, prevents fatty acid oxidation in membrane cell, and also accelerates the secretion of reproduction hormone (Napitu *et al.*, 2013).

Based on above mentioned information, vitamin E is generally effective to induce gonad maturation. However, the vitamin E requirement for broodstocks of sea cucumber is yet unavailable. Thus, it was necessary to conduct this research to understand the role of vitamin E for gonad maturation and reproduction performance on sandfish broodstocks.

MATERIALS AND METHODS

Sandfish used for this experiment were broodstocks where their internal organs were removed by injecting KCl reagent 1 N (evisceration) (Sembiring *et al.*, 2004) to determine the sex ratio and to get the immature stage for all broodstock in the initial experiment. The averages of total length and body weight of broodstocks were 10.95 ± 0.37 cm and 61.12 ± 2.28 g, respectively. The broodstocks were reared in 12 plastic buckets, each with a volume of 150 L (75 cm x 50 cm x 40 cm) and filled with filtered seawater and equipped with an aeration system and water circulation at 1 L/min. Three broodstock were placed into each bucket with the sex ratio of two females and one male. During the experiment, sandfish broodstocks were fed with an experimental diet once daily in the afternoon at 3% of biomass. Uneaten feed, feces, and debris at the bottom of the bucket were siphoned out every day. The experiment was arranged in a completely randomized design with four treatments of different dosages of vitamin E and three replications. The experiment was conducted for 90 days.

Experimental Diets

The main compositions of diets were *Sargassum* sp. and *Ulva* sp. meal with vitamin E added at 0, 150, 300, 450 mg/kg feed (Table 1). Vitamin E used was powder type and contained 500 IU dL- α -tocopherol/g. Feed type was pellet and dried in an oven at 60°C for two hours. Proximate composition of the experimental diet is shown in Table 2.

Collection of Test Parameter Data

Growth measurement was conducted every month. Specific growth rate (SGR) and weight gain (Wg) was calculated using the formula:

$$\text{SGR (\%/day)} = 100 \times \frac{(\ln W_t - \ln W_o)}{t}$$

$$\text{Wg (\%)} = 100 \times \frac{(W_t - W_o)}{W_o}$$

where:

W_o = mean body weight of broodstock at initial of the experiment (g),

W_t = mean body weight of broodstock at the end of the experiment (g), and

t = length of the experiment (day)

At the end of the experiment, all broodstock were dissected in order to observe and analyze its gonad maturation stage. Parameter observed were growth rate as the specific growth rate (SGR), gonad maturation stage, histological structure of gonads, and gonado-somatic index (GSI).

Gonad maturation stage was based on histological observation of gonad samples under a light microscope for each broodstock. The percentage of gonad maturation stage is the number of broodstock reach a certain gonad stage divided with the number of broodstock observed times by 100. Gonad maturation stage was divided into four stages based on the classification by Sembiring *et al.* (2017).

Gonado-somatic index (**GSI**) could be used to determine gonad maturation of sea cucumber and formulation used was: $\text{GW/BW} \times 100$; GW is gonad weight and BW is final body weight (g).

Data Analysis

The effects of treatments on the observed parameters were analyzed by ANOVA, and if the mean is significantly different, it was continued by the Tukey test ($P < 0.05$) using the software R version 3.3.2.

RESULTS AND DISCUSSION

Growth of Sandfish

Growth performance as increasing of body weight and SGR of sandfish fed a diet with different dosages of vitamin E during the experiment were shown in Figure 1 and 2. Mean initial body weight used in this experiment was 61.12 ± 2.28 g and at the end of the experiment, the highest mean body weight was observed with diet-C (300 mg vit. E/kg) at 84.13 ± 1.27 g, followed by diet-D (450 mg vit. E/kg) at 76.83 ± 1.80 g; diet-A (0 mg vit. E/kg) at 70.36 ± 2.21 g, and diet-B (150 mg vit. E/kg) at 67.70 ± 1.12 g.

Table 1. Experimental diets composition for Sandfish broodstock

Ingredients	Experimental diets (g/100 g)			
	A	B	C	D
<i>Sargassum</i> meal	30	30	30	30
<i>Ulva</i> meal	35	35	35	35
Vitamin E	0	0.015	0.03	0.045
Soybean meal	4	4	4	4
Rice flour	18	18	18	18
Fish meal	3	3	3	3
Lap lap meal	5.5	5.485	5.47	5.455
Fish oil	1	1	1	1
Vitamin mix	1	1	1	1
Meneral mix	1	1	1	1
CMC (binder)	1.5	1.5	1.5	1.5

Table 2. Proximate composition of experimental diets

Parameter	Experimental diets			
	A	B	C	D
Ash (% DM)	32	32.2	32.2	31.7
Lipid (% DM)	2.7	3.8	3.5	0.3
Crude protein (% DM)	13.8	14.1	13.8	13.9
Fiber (% DM)	19.9	19.5	17.6	18.2
Nitrogen free extract (% DM)	31.6	30.4	32.9	32.9

Semiring *et al.* (2004; 2015) found that internal organ regeneration of sandfish was completed 30 days after evisceration, gonads started to develop at 40 days, and they could be induced to spawn after 90 days of feeding with experimental diets. Therefore, we ran our gonad maturation experiment for 90 days. Due to the regeneration of internal organ at the initial period of the experiment, most of the internal energy of sandfish was used to develop new organs. After that process was completed then the broodstocks were able to digest the given feed to support its growth. As shown in Figure 1, in the first month of the experiment, bodyweight of broodstock decreased in all treatments. Further, in the second month of feeding, bodyweight of broodstock in all treatment started to recover.

Statistical analysis showed that different dosages of vitamin E in feed have significant effects on specific growth rate and body weight gain of sandfish ($P < 0.05$). The result of the Tukey test showed that SGR of sandfish on treatment-C was significantly higher ($P < 0.05$) compared to treatments-A and B, but was not significantly different with treatment-D. The highest SGR was 0.377% for broodstock fed diet supplemented with 300 mg vit. E/kg diet (Figure 2).

Weight gained of sandfish on treatment-C was significantly different ($P < 0.05$) compare to treatments-A and B, but was not significantly different ($P > 0.05$) compared to treatment-D. However, diet-C did produce the best growth rate (Figure 3). Indeed, diet-C (vitamin E 300 mg/kg) produced the highest specific growth rate and weight gain, indicating that it was the optimal dose to support the growth of the sandfish.

Supplementation of vitamin E in feed gave a positive effect on specific growth rate and weight gained of sandfish. Higher in specific growth rate and weight gained in treatment-C (vitamin E 300 mg/kg diet) indicated that most likely as the optimal dose to support the growth of sandfish.

Body weight gain is affected by the environmental condition, feed, and nutrient composition of feed given during the rearing period. Optimal dietary protein requirement may differ for each species of sandfish. Based on some references, protein requirement for growth of *Apostichopus japonicus* as high as 4.38%-11.2% (Huiling *et al.*, 2004; Bai *et al.*, 2016; Wu *et al.*, 2015). An artificial diet formulated using *Sargassum* sp. meal as the main ingredient with a pro-

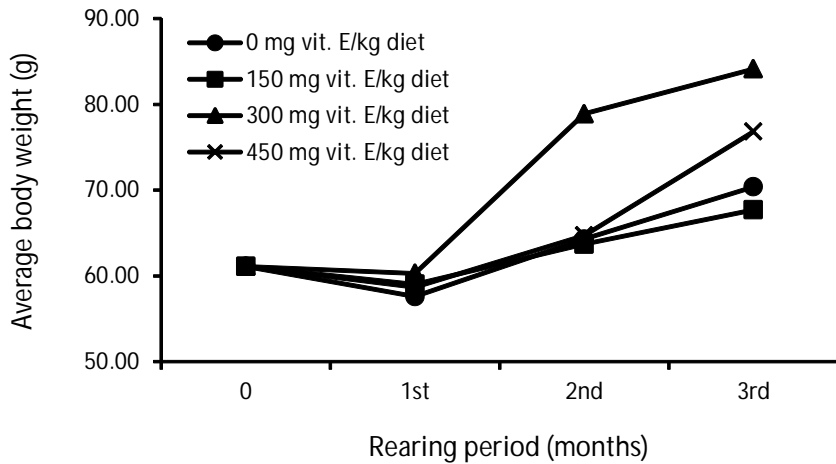


Figure 1. Average body weight of sandfish, *Holothuria scabra* broodstock fed the experimental diet for 90 days of rearing.

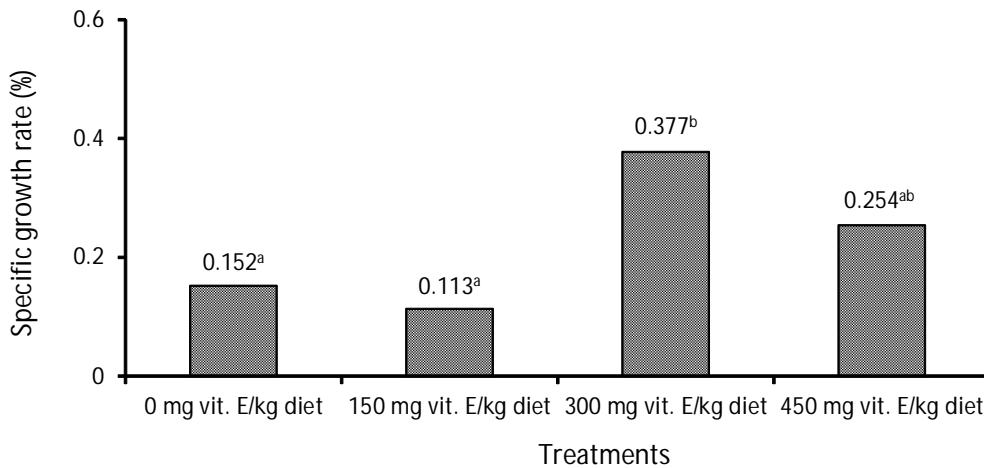


Figure 2. Specific growth rate (SGR, %/day) of sandfish, *Holothuria scabra* broodstock fed with the experimental diet for 90 days of rearing. Values in the figure followed by different superscripts are significantly different.

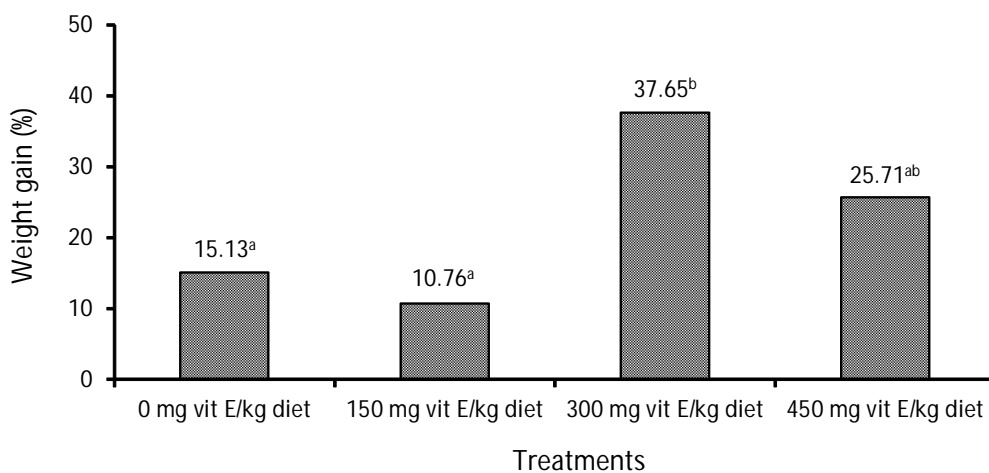


Figure 3. Weight gain of sandfish, *Holothuria scabra* broodstock fed with the experimental diet for 90 days of rearing. Values in the figure with different superscripts are significantly different

tein content of 14.2% was reported able to support good growth for juveniles of *Holothuria scabra* (Giri *et al.*, 2017). In this experiment, sandfish fed with vitamin E-supplemented artificial diet with a protein content of 13%-14% showed good growth performance. We argue that it could gain higher growth if the experiment had used muddy sand as substrate. According to Tizkar *et al.* (2016), vitamin E is absorbed in the intestine and metabolized it in the liver and then stored under the skin during the growth process and in the gonad during gonad maturation process of goldfish.

Gonad Maturation Stage

The result of three months rearing of sandfish fed with diets containing different dosages of vitamin E showed a significant effect on gonad maturation stage (Figure 4). The dosage of vitamin E as high as 300 mg/kg diet and 450 mg/kg diet-induced gonad maturation stage-IV at 11.1%. While dosage of vitamin E as high as 150 mg/kg diet could only induce gonad maturation stage-III (22.2%); stage-II (33.3%), and stage-I (44.4%) and none of them reach stage-IV. Furthermore, a lower dosage of vitamin E in the diet (0 mg/kg diet) only able to support gonad maturation stage-II (33.3%), I (55.5%), and 11.1% of broodstock without any development of their gonads.

Gonad development using diet-C and D were faster than the other treatments because sea cucumber broodstock responded positively to vitamin E and utilized it for the gonad maturation process. The vitellogenesis process (yolk formation in the oocyte) requires nutrients and vitamin E, which further stimulates the formation of egg and ovarian maturation (Li & Zhang, 2017). Asaikkutti *et al.* (2016) and Ni *et al.*

(2015) reported that vitamin E could function as an antioxidant as well as a coenzyme in the reaction from fatty acid to cholesterol. Cholesterol stimulates the formation of reproduction hormone 17-β estradiol which is needed in vitellogenesis (Subramoniam, 2017).

Histological analysis showed there were different oocyte stages found in each gonad sample from each treatment (Figure 5) of which the larger oocytes increased the value of the gonado-somatic index. The results of histological analysis showed that gonad maturation stage-I has an average diameter of oocyte of $41.7 \pm 8.8 \mu\text{m}$; gonad maturation stage-II ($124.8 \pm 11.0 \mu\text{m}$); gonad maturation stage-III ($188.1 \pm 8.2 \mu\text{m}$); and gonad maturation stage-IV ($233.0 \pm 8.4 \mu\text{m}$).

Gonado-Somatic Index

The average value of GSI from the highest to the lowest was 12.21% in treatment-C; 8.78% in treatment-D; 8.26% in treatment-B; and 6.83% in treatment-A (Figure 6).

Based on statistical analysis at 95% of the confidential level, GSI of sandfish broodstock fed with the diet of treatment-C was significantly different compared to that of treatment-A and B ($P < 0.05$) but was not significantly different compared to that of treatment-D ($P > 0.05$). The highest value of GSI was achieved by sandfish of treatment-C with a dosage of vitamin E 300 mg/kg diet. The increase of GSI and percentage of gonadal maturation stage (GMS) IV in treatment-C and D during three months of feeding experiment was due to optimal content of vitamin E in the diet to increase the availability of fatty acid. Availability of fatty acid could induce vitellogenin for-

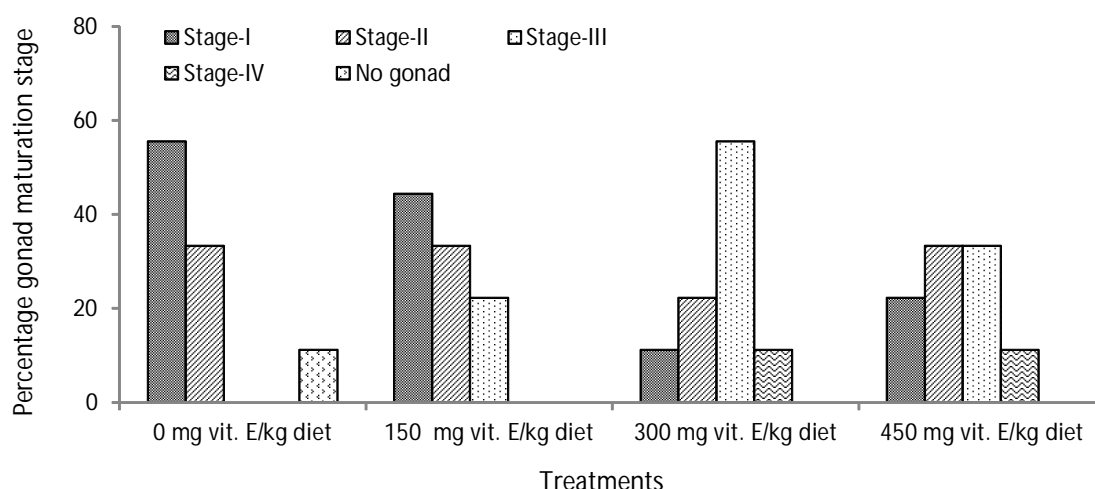


Figure 4. Percentage gonad maturation stages of sandfish, *Holothuria scabra* broodstock fed the experimental diet for 90 days of rearing.

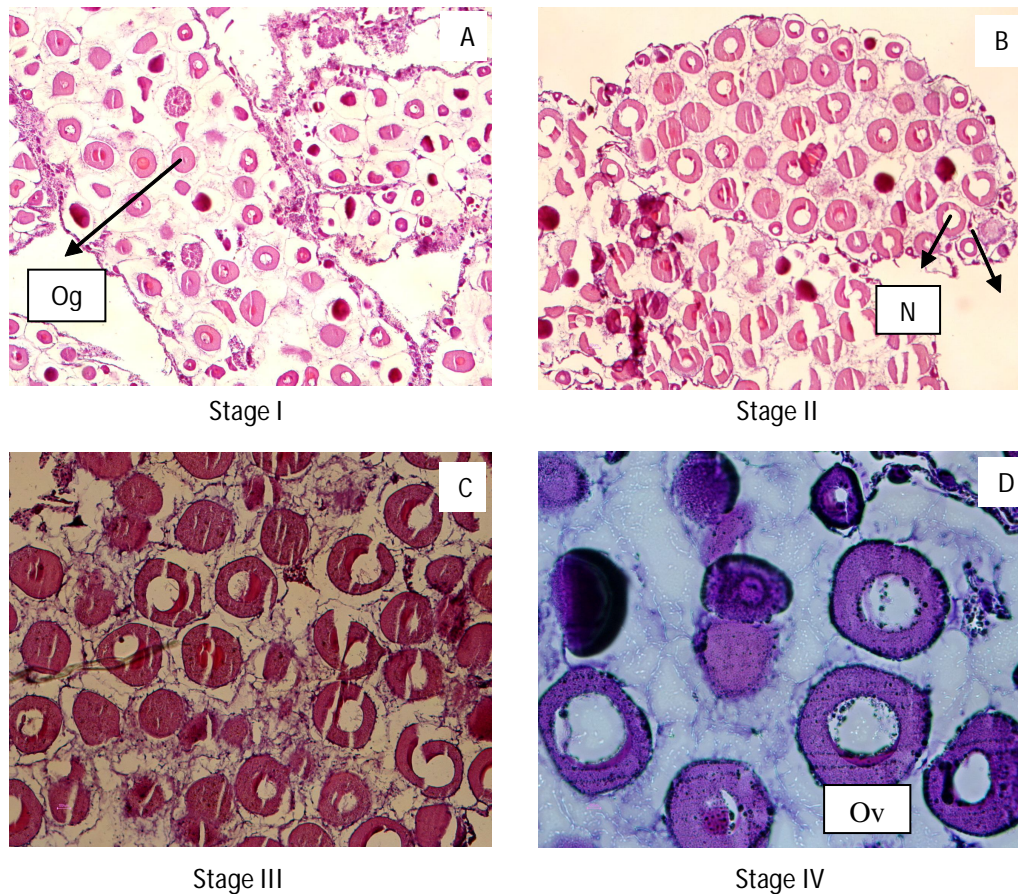


Figure 5. Gonad development on sandfish, *H. scabra* broodstock after 90 days of rearing (magnification 10 x 10 for A & B and 40 x 10 for C&D).

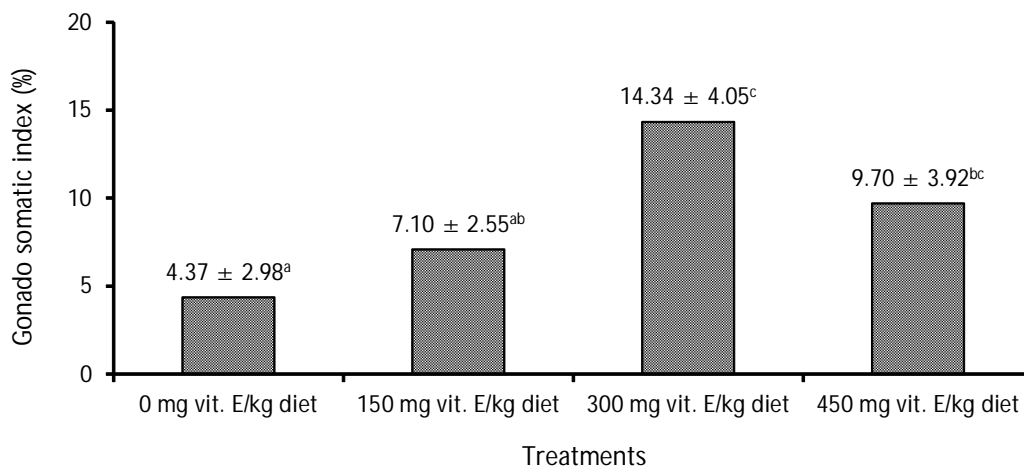


Figure 6. Gonado-somatic index (GSI) values of sea cucumber, *Holothuria scabra* broodstock fed with the experimental diet for 90 days of rearing. Values in the figure with different superscripts are significantly different.

mation in the liver during the vitellogenesis process and deliver it into the ovary to form egg cells (Reading & Sullivan, 2011). During vitellogenesis, the ovary will absorb vitellogenin and maximize the accumulated vitellogenin (Williams *et al.*, 2014). Optimiza-

tion of vitellogenin formation will accelerate the formation and development of gonad, leading to increased gonad weight and subsequently, the percentage of more mature gonad sandfish will increase.

CONCLUSION

The use of vitamin E as a diet supplement showed promising results. The 300 mg/kg dose appeared to be the optimal dose, due to improvement of growth and inducing gonad maturation more than zero or 150 mg/kg diets, and more than 450 mg/kg. The optimum rearing conditions could be achieved by providing a muddy sand substrate which is a suggestion for future experiment to improve growth rates and enhance spawning success in *H. scabra* receiving vitamin E supplements.

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