

# The Potency of Sandfish (*Holothuria scabra*) as a Source of Natural Aphrodisiacs

Etty Riani, Endang Gumbira-Said, Kashwar Syamsu, Kustiariyah, Kaseno, Muhammad Reza Cordova

**Abstract**—Sandfish is one of marine biota that has a biomedicine (bioactive compound) potency. People in Gorontalo Province, Indonesia, have been sandfish as an aphrodisiac for men as it is believed that sandfish has a steroid hormone potency. This research aims at studying using the steroid hormone potency from every fraction of sandfish (meat and innards) and its activity of male reproduction (rooster) as an aphrodisiac. Steroid extraction was done using Touchstone and Kasparow method, and then it was utilized to study the effectiveness of bioassay of rooster. This research had five treatments and was done in complete randomized design. Based on Lieberman-Burchard and bioassay test, the author found that sandfish extract contains steroid hormone. Sandfish extract was able to enrich testosterone and cholesterol concentration in blood serum; fastening secondary reproduction characteristics of the rooster, and increasing growth as well as improving rooster's comb. Therefore, sandfish steroid is potential to be used as an aphrodisiac for men.

**Keywords**—Aphrodisiac, sandfish, secondary reproduction characteristic, steroid.

## I. INTRODUCTION

INDONESIA has a great marine resources potency and biodiversity. Those marine resources have many benefits that can be utilized as food, medicine, cosmetic, etc. [1]. One of marine biota that has been known having bio active compound that can be used for various uses is sandfish. Sandfish is one of fishery commodities with significant economic value, has been traded in dry form, mostly without added value, for all this time. Sandfish is traditionally believed being able to cure some diseases, and improve human health. This biota is also recognized to contain active compounds that are utilized in pharmaceuticals and healthcare.

Literature study showed that most research about sandfish were still limited to its farming techniques [2], ecology and distribution potency [3]-[5] processing step [6], antibacterial and antifungal activities of sandfish *Holothuria* sp. [7] and [8], antibacterial activity of sandfish *Cucumaria frondosa* [9], antifungal activity of sandfish *Psolus patagonicus* [10], isolation of arginine and kinase enzyme from sandfish *Stichopus japonicas* [11], activity of A amyloid serum of

sandfish *Holothuria glaberrina* [12], glycoside structure of sandfish *Stichopus mollis* [13], and isolation of fucan sulfate from sandfish *Stichopus japonicas* as osteoclastogenesis inhibitory factor [14]. Meanwhile the steroid active compound research for natural aphrodisiac production from sandfish is still relatively at minimum information.

Sandfish also has arginine kinase enzyme active compound, an amyloid serum, glycoside structure and fucan sulfate as osteoclastogenesis inhibitory factors. Some active compounds of sandfish had been patented, such as triterpene from *Acacia victoriae* as anti-tumor (US Patent 7.105.186), glycoside component as anti-cancer (US Patent 7.144.867) and natural coloring substance from *Holothuria scabra* (US Patent 6.956.122). However, study of active compounds that can be used as biopharmaceutical substances such aphrodisiac (male vitality enhancer) has not been reported. However according to Gorontalo people (Gorontalo Province, Sulawesi, Indonesia) sandfish can be used to increase male vitality. Therefore, this research observes the potency of sandfish as a natural resource of aphrodisiac.

This research aims at studying steroid hormone potency from every fraction of sandfish (meat and innards) and its activity of male reproduction (rooster) as aphrodisiac.

## II. RESEARCH METHOD

Sandfish used in this research was taken from the waters of Marine Culture Center Lampung, and there were also material and equipment to extract steroid. Touchstone and Kasparow method (1970 in [15]) was used to extract steroid. Steroid from sandfish, either from meat or innards, were identified by Lieberman Burchard test and steroid separation was done by TLC technique. Isolated steroids were activity tested, and then identified to its characteristic which includes molecular weight, composition, effectiveness, and stability measurements. Furthermore, the stability to damage chance caused by storage and environment condition was also tested. Therefore the active compounds were tested again after 1, 2, and 4 weeks of storage times. Stabilities of these active compounds were also tested at different temperatures, which was 4° and 25°C (room temperature).

- 1) Steroid extract that had been obtained further was effectiveness/bioassay tested in male chick. Chick was ND (New Castle Disease) vaccinated, weighed, and measured for the length, width, and height of comb. The chicks were cared in cages, each cage contained five chicks which each had the same treatment.
- 2) The chicks were body weighed each day and measured for its length, width, and comb height every two days. The

E. Riani is with the Department of Aquatic Resources Management; Bogor Agricultural University, Indonesia (corresponding author to email: etty\_riani\_harsono@yahoo.com).

E. Gumbira-Said and K. Syamsu are with the Department of Agro Industrial Technology, Bogor Agricultural University, Indonesia.

Kustiariyah is with the Department of Aquatic Product Technology, Bogor Agricultural University, Indonesia.

Kaseno is with the Agency for The Assessment and Application of Technology, Indonesia.

M.R. Cordova is with the Center for Oceanography and Marine Technology, Surya University, Indonesia.

blood was taken from neck on day 31 for cholesterol and triglyceride determination, and the chick was cut to be taken the comb, liver, spleen, bursa fabricus, and testis, and then these were weighed.

The experiment was designed to obtain data of sandfish steroid effect to body weight, testis weight, spleen, liver, cholesterol concentration and blood testosterone in male chick. The research design on bioassay was Completely Randomized Design [16]. The treatment was the provision of basal ratio once daily with:

1. No steroid and fed with 0.5ml of corn oil as negative control
2. Fed with 0.5ml of dry sandfish steroid 0.4g/ml
3. Fed with 0.5ml of frozen sandfish steroid 0.4g/ml
4. Fed with 0.5ml of methyl testosterone 0.4mg/ml (positive control)
5. Fed with 0.5ml of commercial aphrodisiac 0.4mg/ml (positive control)

The design used in bioassay step was a Completely Randomized Design (CRD). Mathematical model of the design is presented below [16].

$$Y_{ij} = \mu + P_i + \varepsilon_{ij} \quad (1)$$

$Y_{ij}$  = the observation value (response) from factors of the-i treatment and the-j repetition

$\mu$  = the real average value

$P_i$  = the-i treatment effect

$\varepsilon_{ij}$  = the error effect to the-i treatment and the-j repetition

### III. RESULT

#### A. Steroid Extraction

Steroid extraction was conducted based on method reported by Touchstone and Kasparow (1970) in [15]. The extraction of 1kg dry sandfish meat and 1kg dry innards resulted yields as presented in Table I.

#### B. Identification of Steroid Presence and Fractionation

Identification of steroid presence was conducted to meat and innards of sandfish through qualitative test by Lieberman Burchard test. The test result can be seen in Table II.

These results were consistent to the color test results which showed positive extraction result containing steroid. Thus it can

be concluded that meat and innards which are sandfish waste contain steroid hormone that can be used for various purposes such as aphrodisiac and sex reversal.

TABLE I  
YIELD OF SANDFISH STEROID EXTRACTION USING ACETONE

Material (1 kg)	Yield of steroid crude extract (g)
Dry sandfish meat	8.16
Dry innards	17.96
Frozen fresh sandfish meat	12.96
Frozen fresh innards	21.28

TABLE II  
IDENTIFICATION RESULT OF STEROID PRESENCE IN SANDFISH

No.	Sample	Observation (qualitative)
1	Wet meat	+
2	Dry meat	+
3	Wet innards	+++
4	Dry innards	++

Characteristics of steroid sandfish extract in addition to its biological activity (androgenic) to male chick were as follows:

- a. The color of steroid extract from sandfish meat was greenish white, while innards extract's is brownish white.
- b. pH of extracts tend to be neutral, with a range of 7.3-7.6
- c. Steroid compound of sandfish more soluble in bipolar solvent (acetone) than in polar solvent (water and methanol) and non-polar solvent (dichloromethane/DCM)
- d. Active compounds of sandfish extract remain stable after low temperature storage ( $\pm 10^\circ\text{C}$ ) for 10 months
- e. Sandfish extract has antibacterial to *Staphylococcus aureus* (gram positive bacteria). Diameter of clear zone was formed by 14mm. But did not inhibit the growth of *E. coli*, *Pseudomonas aeruginosa* (both are gram-negative bacteria) and *Bacillus subtilis*.
- f. Extract of sandfish can inhibit *Candida maltosa* growth.

#### C. Bioassay in Chicken

Effect of sandfish extract to the weight of liver, spleen, and bursa fabricus are as follows. Fig. 1 presents the average of liver weights of test animals. Average weight of male chick spleens that were treated with dry extract, can be seen in Fig. 2, while the average value of bursa fabricus weights can be seen in Fig. 3.

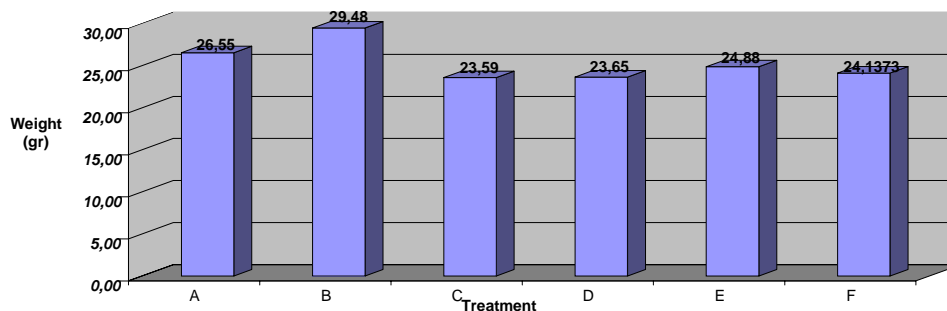


Fig. 1 Average weights of male chick liver A. Negative control, B. Dry sandfish extract, C. Wet sandfish extract, D. Positive control, E. Commercial steroid, F. Dry sandfish powder

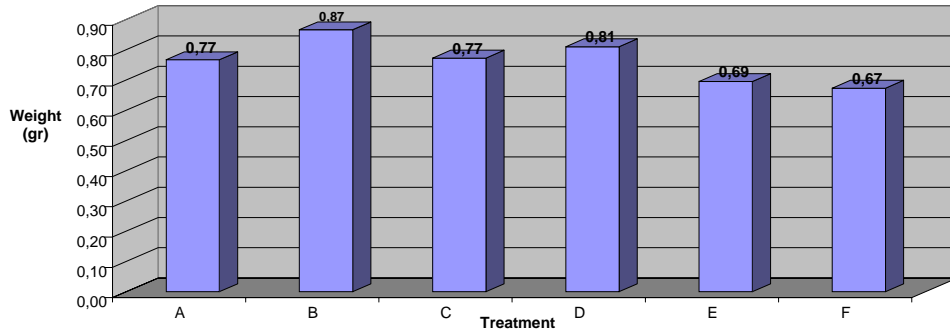


Fig. 2 Average weights of male chick spleen A. Negative control, B. Dry sandfish extract, C. Wet sandfish extract, D. Positive control, E. Commercial steroid, F. Dry sandfish powder.

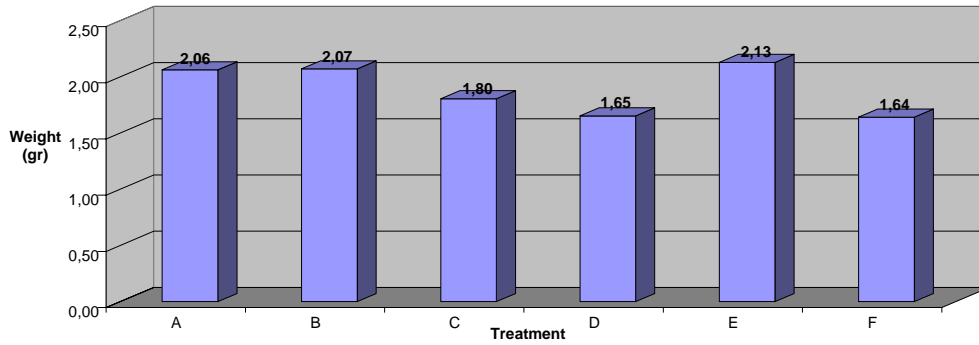


Fig. 3 Average weights of male chick bursa fabricius A. Negative control, B. Dry sandfish extract, C. Wet sandfish extract, D. Positive control, E. Commercial steroid, F. Dry sandfish powder

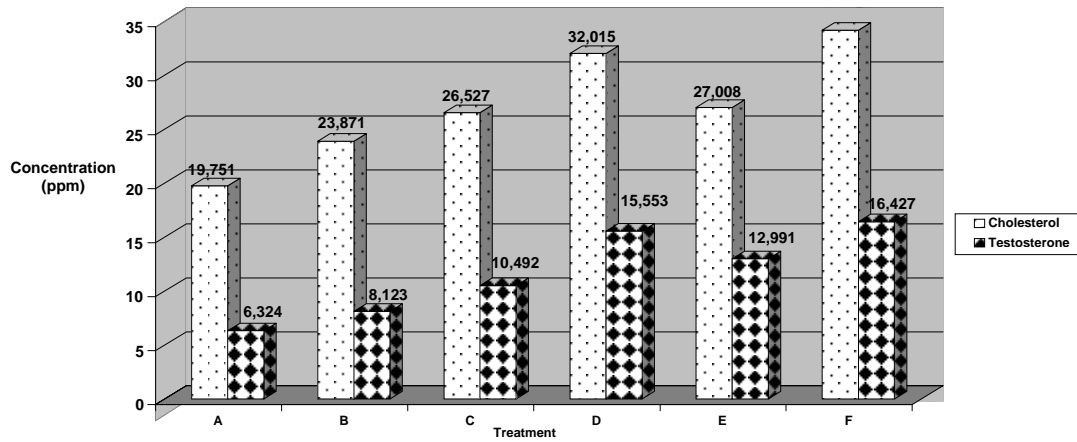


Fig. 4 Testosterone and cholesterol concentration in blood serum of male chicks

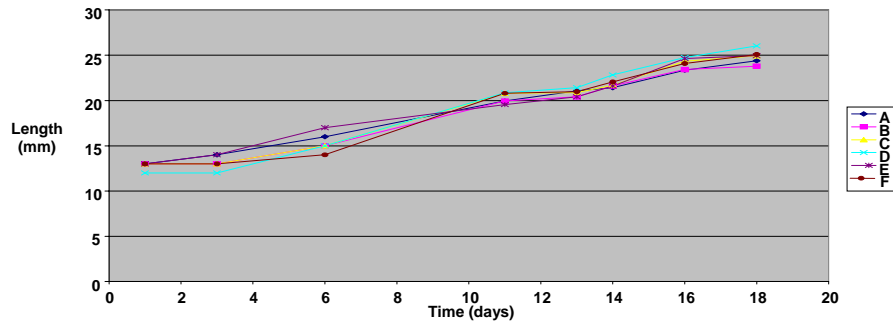


Fig. 5 The increase of comb length in male chicks A. Negative control, B. Dry sandfish extract, C. Wet sandfish extract, D. Positive control, E. Commercial steroid, F. Dry sandfish powder

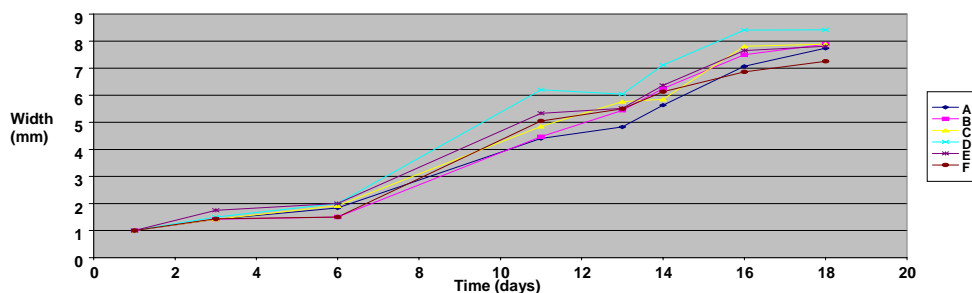


Fig. 6 The increase of comb width in male chicks A. Negative control, B. Dry sandfish extract, C. Wet sandfish extract, D. Positive control, E. Commercial steroid, F. Dry sandfish powder

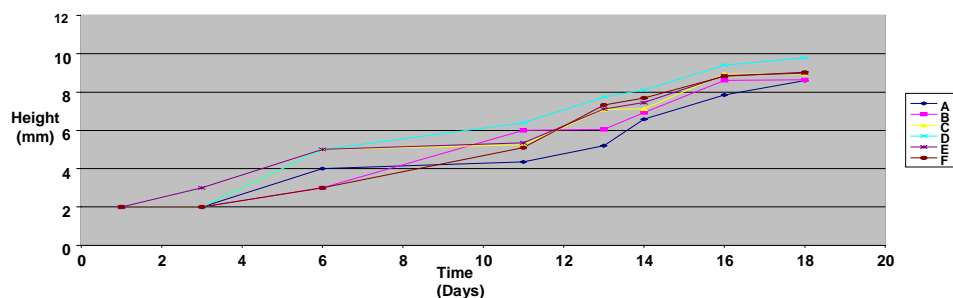


Fig. 7 The increase of comb height in male chicks A. Negative control, B. Dry sandfish extract, C. Wet sandfish extract, D. Positive control, E. Commercial steroid, F. Dry sandfish powder

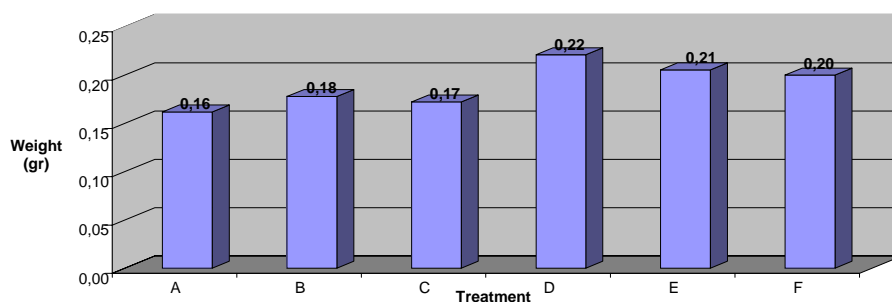


Fig. 8 The average of testis weight in male chicks A. Negative control, B. Dry sandfish extract, C. Wet sandfish extract, D. Positive control, E. Commercial steroid, F. Dry sandfish powder

#### D. Effect of Extract to Testosterone and Cholesterol Concentration

Cholesterol concentrations in blood serum of male chicks that were treated with sandfish powder are shown in Fig 4.

#### E. Effect of Extract to Length, Width, and Height of Comb

The comb development in male chick that was treated with testosterone, either natural or synthetic, was lead to the comb growth that was shown in Figs. 5-7. The development of width comb can be seen in Fig 7.

#### F. Effect of Extract to Testis Weight

The effect of extract to testis weight is shown in Fig. 8.

### IV. DISCUSSION

#### A. Natural Steroid Potencies of Sandfish

The results of steroid extraction conducted based on Touchstone and Kasparow method (1970) in [15], showed that both the meat and waste (innards) of sandfish can produce

steroid crude extract yield (Table I). This condition was reinforced by the result of qualitative test (Lieberman Burchard) to identify the steroid presence in sandfish, which indicates the presence of steroid in meat and waste of sandfish (Table II). Identification result of steroid presence shows that steroid in waste (innards) identified more strongly than steroid in meat. This shows that sandfish is potential to be source of natural steroid compound. Table I shows that steroid crude extract obtained from waste (innards) have much higher yield, and identification result also shows that steroid of waste (innards) known to be stronger than the meat. This condition indicates that waste (innards) of sandfish has a higher potency to produce steroid than the meat, so that the opportunity to utilize and process sandfish wastes and convert the worthless things even tend to pollute the environment, into things with high economic value, is higher.

The characteristics of sandfish steroid extract show steroid characteristic in general ways, but the result of characteristic test also represents the presence of some advantages than other

natural steroids. In this case, the steroid of sandfish remained stable despite of having storage at low temperature ( $\pm 10^{\circ}\text{C}$ ) for 10 months. Besides, steroid extract of sandfish also has antibacterial activity against *Staphylococcus aureus* (gram-positive bacteria) and inhibits the growth of *Candida maltosa*. Thus, steroid extract of sandfish also has advantages over other natural steroids, especially in the case of having antibacterial activity, so steroid of sandfish has a save life that is more durable.

*B. Potency of Sandfish Steroid as Natural Aphrodisiac, Feed N Uptake by in vivo and Its Effect to Body Weight*

In this experiment of feed N uptake by in vivo, the analysis result of N concentration in chick feces was obtained and is shown in Fig. 9. As with other animals in general, the effectiveness of feed N uptake in animals can be seen by its body weight development. However, the delivery of drug compounds in organism, such as chick, can affect (tends to decrease) body weight of chick. This was due to several types of drugs that can lead to impaired metabolism, for example, can reduce absorption of protein, some vitamins, and other nutrients.

The development of body weight of tested animal that resulted in this research is presented in Fig. 10. The highest average of body weight gain obtained from the treatment with

dry sandfish extract, which was equal to 57.62g. The body weight gains of chicks that were treated by sandfish extract were higher than body weight gain of ones in control group, which was equal to 56.43g. Meanwhile the lowest average of body weight gain was obtained from the treatment of dry sandfish powder, that was 50.48g. While in the positive control, both by using methyl testosterone and commercial aphrodisiac, showed a body weight gain, but the increase tend to be low, those were 52.38 and 52.86g respectively.

The effect of sandfish to the body weight of chick and its influence to the organs play a role in immune system were determined in this study to see toxicity representation of sandfish steroid extract. In this case if a given compound was toxic, the compound would be a foreign substance (antigen) and could lead to the swelling of liver, spleen, and bursa fabricius.

Bioassay test result, the effect of sandfish extract to the liver weight of male chick, showed that the highest average value occurred in dry extract treatment, which was equal to 29.48g, while the lowest was in the positive control (dry extract giving) by 23.65g. The result of calculation of ratio of liver weight to body weight, that the highest average value was in dry extract treatment by 3.15; while the lowest was in the positive control by 2.78.

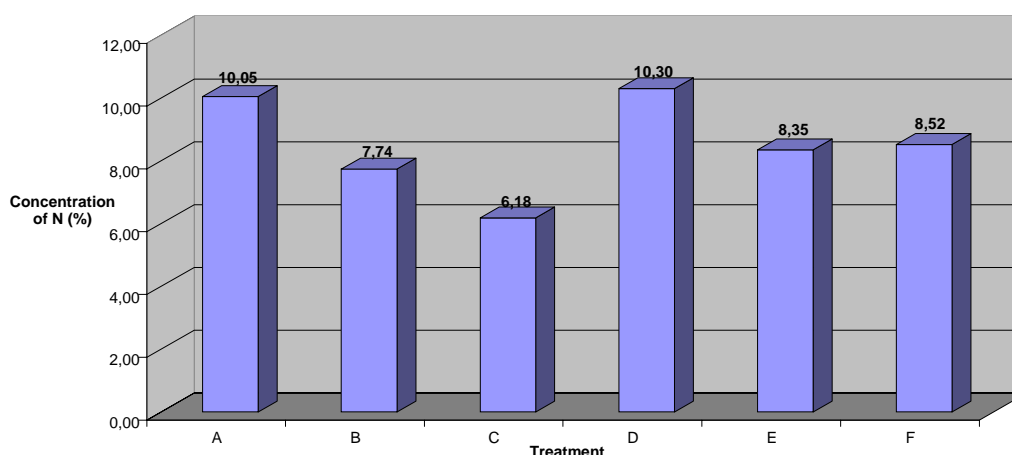


Fig. 9 Concentration of N in male chick faeces A. The negative control, B. Dry sandfish extract, C. Wet sandfish extract, D. The positive control, E. Commercial steroid, F. Dry sandfish powder

The highest average of spleen weight of male chick was in dry extract treatment, which was equal to 0.87g, while the lowest was in dry sandfish powder, that was 0.69g (Fig. 2). The ratio calculation of spleen weight to body weight showed that highest average value was in the positive control by 0,10 and the lowest was in the negative control, commercial aphrodisiac and dry sandfish powder treatment, which was equal to 0,08 respectively.

The highest average value of bursa fabricius was in commercial aphrodisiac treatment, that was 2.13g, while the lowest was in dry sandfish powder treatment, which was 1.64g (Fig. 3). In this study, bursa fabricius of chick that was commercial aphrodisiac treated indicated tend to be swollen and larger in size. This was also be seen from the highest

average value of ratio of bursa fabricius weight to body weight in commercial aphrodisiac treatment, that was equal to 0.24.

Based on the analysis of variance to body weight, liver weight, spleen weight, and bursa fabricius weight, giving sandfish extract did not significantly affect body weight, liver weight, spleen weight, and bursa fabricius weight. This is presumably because bioassay performed in too short way, so it did not give significantly affect yet to the body weight, liver weight, spleen weight, and bursa fabricius weight. However, there is a tendency that commercial aphrodisiac lead to bursa fabricius swelling and this is also proved by the weight of bursa fabricius and ratio of bursa fabricius weight to body weight those are the highest. This condition is one thing that should be considered seriously to its possibility of negative impact to

immune system organs, given the synthetic steroid is often regarded as hazardous foreign substance (antigen) to the body. This is consistent with the result of short communication (2007) carried out by [17] that concluded methyl testosterone is a carcinogenic and teratogenic substance, and also [18] that give result of uncontrolled growth and congenital defect occurrences in prawns treated by methyl testosterone.

The highest cholesterol concentration in blood serum of chicken occurred in the treatment of dry sandfish powder, that was 34,158ppm, while the lowest was in the negative control by 18,761ppm (Fig. 4). Testosterone in blood serum is known to be related to cholesterol, because cholesterol is testosterone precursor [19]. The highest testosterone level also occurred in dry sandfish powder treatment, that was 16,427ppm, and the lowest was in the negative control with 6,324ppm. This happened because sandfish extract also stimulates new synthesis of testosterone from cholesterol besides providing testosterone [20], so that concentration of testosterone achieves optimum limit and changes the behavior of male chick through sexual maturation, such as crowing and competing, and also the development of comb like in a rooster.

In general, the development of comb in male chick is associated with the sexual development or maturation, because the comb is one of sex accessories glands in male. Thus the growth and development of comb only occurs naturally in rooster, while it never does in male chick. The comb growing is an indicator of sexual maturity or testosterone producing in rooster. In this study, the chicks that were given both natural and synthetic testosterone resulted in the growth of comb (Figs. 5-7). The highest length of comb occurred in the positive control, which was 26.03mm, and the lowest was in the treatment of dry extract by 23.80mm. Thus it is concluded that dry sandfish extract contains testosterone hormone recognized by receptors inside male chick's body in test [19]. The highest development of width comb also happened in the positive control, that was 8.42mm and the lowest was in dry sandfish powder treatment by 7.26mm (Fig. 7). The fastest development of height comb in tested animal occurred in the positive control, which was 9.80mm, and the lowest was in the negative control with 8.60mm in height (Fig. 7).



Fig. 10 Body weight gain in male chicks A. The negative control, B. Dry sandfish extract, C. Wet sandfish extract, D. The positive control, E. Commercial steroid, F. Dry sandfish powder

Based on analysis of variance, the giving of sandfish extract significantly affected the width and height of comb ( $\alpha=0.01$ ), but did not significantly affect the length of comb. The result of analysis of variance showed that the width of comb in the positive control (methyl testosterone) was significantly different from other treatments ( $\alpha=0.05$ ). Commercial aphrodisiac and wet sandfish extract (C treatment) were not significantly affected the width of comb, but those were significantly different from the negative control. The giving of sandfish extract, either dry or wet extract, in male chick was significantly different from the negative control ( $\alpha=0.05$ ), but not significantly different among those treatments.

The effect of extract to testis weight can be seen in Fig. 8. It shows that the giving of crude extract of sandfish steroid, methyl testosterone, and commercial aphrodisiac affected the development of testis in tested animal.

Based on primary and secondary reproduction characteristics data, it appears that even though sandfish extract did not give the highest value, but the sandfish extract contains testosterone in a sufficient number enough to stimulate the development of sexual maturation and functional of testis. It shows that sandfish steroid is potential as natural aphrodisiac which is safe and environmentally friendly and also supports the zero waste action because it could be produced from innards waste of

sandfish, and according to [19] the residual excretion will not cause environmental damage.

#### V. CONCLUSIONS

1. The sandfish extract provides the enrichment of testosterone and cholesterol concentration in blood serum and the occurrence of fastening secondary reproduction characteristic in male chick.
2. Steroid compound of sandfish was significantly affected the growth of width and height of male chick comb.
3. Steroid compound of sandfish was significantly affected the concentration of cholesterol and testosterone in blood serum in male chick
4. Sandfish is potential as a natural aphrodisiac which is safe and environmentally friendly.

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#### REFERENCES

- [1] R. Dahuri, "New Paradigm of Indonesian Development Base on Marine (Unpublished work style)," Special Scientific Speech in Faculty of Fisheries and Marine Science, Bogor Agricultural University, Bogor, 2003. (in Indonesian).
- [2] A. Aziz, "Commercial Sea Cucumber Research Status in Indonesia," *Oseana*, 1997, vol 22(1), pp. 9 -19. (in Indonesian)
- [3] S. Nuraini and S. Wahyuni, "Sea Cucumber Fisheries in Karimun Jawa Archipelago and Jepara," *Research Journal. Marine Fisheries*, 1989, vol52, pp. 77-81. (in Indonesian)
- [4] A. Mercier, S. Battaglene and J. F. Hamel, "Daily Burrowing Cycle and Feeding Activity of Juvenile Sea Cucumber *Holothuriascabra* in Response to Environmental Factors," *Journal of Experimental marine Biology and Ecology*, 1999, vol239, pp. 125-156
- [5] S. Dżeroski and D. Drumm, "Using Regression Trees to Identify the Habitat Preference of the Sea Cucumber (*Holothurialeucospilota*) on Rotonga, Cook Islands," *Ecological Modelling*, 2003, vol 70, pp. 219-226
- [6] S. Wibowo, E. Yunizal, M. D. Setiabudi, Erlina and Tazwir, "Technology of Sea Cucumber (Holothuridae) Handling and Processing, Jakarta: *IPPL Slipi*, 1997. (in Indonesian)
- [7] M. A. Kaswandi, H. H Lian, S. Nurzakiah, B. H. Ridzwan, S. Ujang, M. W. Samsudin, S. Jasnizat and A. M, Ali, "Crystal Saponin from Three Sea Cucumber Genus and Their Potential as Antibacterial Agents," *9th Scientific Conference Electron Microscopic Society*, 12-14 Nov. 2000, Kota Bharu, Kelantan, 2000, pp. 273—276.
- [8] H. H. Lian, S. N. Weng, M. S. M. Yassin, M. A. Kaswandi and B. H. Ridzwan, "Antifungal Activities of Lipid Extract from Sea Cucumber *Holothuria tubulosa* Against *Saccharomyces cerevisiae*," *7th Asia Pacific Electron Microscopy Conf.* 26—30 June, Singapore. 2000, p. 316.
- [9] T. Haug, A. K. Kjuul, O. B. Styrvoid, E. Sandsdalen, O. M. Olsen and K. Stensvag, "Antibacterial Activity in *Strongylocentrotus droebachiensis* (Echinoidea), *Cucumaria frondosa* (Holothuroidea) and *Asterias rubens* (Asteroidea)," *Journal of Invertebrate Pathology*, 2002, vol 81, pp. 94-102
- [10] A. P. Murray, C. Muniain, A. M. Seldes and M. Maier, "Patagonicoside A: a Novel Antifungal Disulfated Triterpene Glycoside from the Sea Cucumber *Psoluspatagonicus*," *Tetrahedron*, 2001, vol57, pp. 9563-9568.
- [11] S. Y. Guo, Z. Guo, Q. Guo, B. Y. Chen and X. C. Wang, "Expression, Purification and Characterization of Arginine Kinase from the Sea Cucumber *Stichopus japonicus*," *Protein Expression and Purification*, 2003, vol29, pp. 230-234.
- [12] P. G. S. Cardona, C. A. Berrios, F. Ramirez and J. E. G. Arrarás, "Lipopolysaccharides Induce Intestinal Serum Amyloid A Expression in the Sea Cucumber *Holothuriaglaberrima*," *Development and Comparative Immunology*, 2003, vol27, pp.105-110.
- [13] G. Moraes, P. C. Norchote, V. I. Kalinin, S. A. Avilov, A. Silchenko, P. S. Dmitrenok, V. A. Stonik and V. Levin, "Structure of the Major Triterpene Glycoside from the Sea Cucumber *Stichopus malleus* and Evidence to Reclassify this Species into the New Genus *Australostichopus*," *Biochemical Systematic and Ecology*, 2004, vol 32, pp. 637- 650.
- [14] Y. Kariya, B. Mulloy, K. Imai, A. Tominaga, T. Kaneko, A. Asari, K. Suzuki, H. Masuda, M. Kyosashima and T. Ishii, "Isolation and Partial Characterization of Fucan Sulfates from the Body wall of Sea Cucumber *Stichopus japonicus* and their ability to inhibit Osteoclastogenesis," *Carbohydrate Research*, 2004, vol339, pp. 1339-1346.
- [15] I. D. Riris, "Steroid in Green Mussels (Thesis)". Bogor: Bogor Agricultural University, 1994. (in Indonesian)
- [16] R. G. D. Steel and J. H. Torrie "Statistic Principle and Procedure," Jakarta: Gramedia Pustaka Utama, 1995. (in Indonesian)
- [17] G. Vogt, "Exposure of the Eggs to 17alpha-methyl Testosterone Reduced Hatching Success and Growth and Elicited Teratogenic Effects in Postembryonic Life Stages of Crayfish," *Aquat Toxicol*, 2007, vol 85(4), pp. 291-296.
- [18] E. Riani, "Effectivity of Sandfish Extract, Formulated to Giant Prawn Masculinize," *Bionatura*, 2010, Vol 12 (3), pp. 145 – 154. (in Indonesian)
- [19] G. G. Ying, R. S. Kookana and Y. Z. Ru (2002) "Occurrence and Fate of Hormone Steroids in the Environment," *Journal Environment International*, 2002, Vol28, 545–551.
- [20] E. Riani, K. Syamsu, Kaseno, S. Nurjanah, K. H. Dewi and Kustiariyah "Sandfish Steroid as Natural Aphrodisiac for Man (Unpublished work style)," Report of the Post Graduate Research Grant of 2007, Bogor Agricultural University, Bogor, 2007. (in Indonesian).