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SEX REVERSAL OF NILE TILAPIA (OREOCHROMIS NILOTICUS) THROUGH IMMERSION IN METHANOL EXTRACT OF SEA URCHIN (DIADEMA SETOSUM) GONAD AT A DOSE OF 8 MG/L

Sex Reversal Ikan Nila (*Oreochromis Niloticus*) Dengan Perendaman Dalam Ekstrak Metanol Gonad Bulu Babi *(Diadema Setosum)* Dosis 8 Mg/L

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ABSTRACT

One type of fish that is resistant to low quality water is Nile tilapia with the name Oreochromis niloticus. Sexual dimorphism in Nile tilapia shows that males grow faster than females, making monosex male tilapia more advantageous. Immersion can provide natural steroid hormones to increase the number of male individuals. *Diadema setosum* or sea urchins are known to contain bioactive compounds from the steroid, flavonoid, and saponin groups. The purpose of this study was to determine how the administration of sea urchin gonad extract at a dose of 8 mg/L to male tilapia affects their growth. This study was conducted during various soaking times. In this study, a completely randomized design (CRD) was applied with four treatments and three replications. The dosage was 8 mg/L, and the soaking times were 0 hours (control), 12, 18, and 24 hours. SPSS 16 software was used in data processing with analysis of variance (one way Anova) and Least Significant Difference at the 5% level ($\alpha > 0.05$). The results showed that immersion time in sea urchin gonad extract solution at a dose of 8 mg/L did not affect sperm production. Within twelve hours, the immersion successfully changed the sex of the fish to male by 38.4%. The survival rate of tilapia (Oreochromis niloticus) larvae was not affected by immersion time.

Key words : Sea Urchin Gonads; Sex reversal; Tilapia.

ABSTRAK

Salah satu jenis ikan yang tahan terhadap air berkualitas rendah ialah ikan Nila dengan nama laitn *Oreochromis niloticus*. Dimorfisme seksual pada ikan nila memperlihatkan bahwasanya ikan jantan tumbuh lebih pesat apabila dibandingkan dengan ikan betina, sehingga ikan jantan monoseks lebih menguntungkan. Perendaman dapat memberikan hormon steroid alami untuk meningkatkan jumlah individu jantan. *Diadema setosum* atau bulu babi, diketahui mempunyai senyawa bioaktif dari kelompok steroid, flavonoid, dan saponin. Penelitian ini mempunyai tujuan untuk mengetahui bagaimana pemberian ekstrak gonad bulu babi dosis 8 mg/L pada ikan nila jantan mempengaruhi pertumbuhannya. Penelitian ini dilakukan selama berbagai waktu perendaman. Pada penelitian ini, rancangan acak lengkap (RAL) diterapkan dengan empat perlakuan dan tiga ulangan. Dosisnya adalah 8 mg/L, dan waktu perendaman adalah 0 jam (kontrol), 12, 18, dan 24 jam. Software SPSS 16 digunakan dalam pengolahan data dengan analisis sidik ragam (one way Anova) dan Beda Nyata Terkecil dengan taraf 5% ($\alpha > 0{,}05$). Hasil penelitian memperlihatkan bahwa waktu perendaman dalam larutan ekstrak gonad bulu babi dengan dosis 8 mg/L tidak memengaruhi produksi sperma. Dalam waktu dua belas jam, perendaman berhasil mengubah jenis kelamin ikan menjadi jantan sebesar 38,4%. Tingkat kelangsungan hidup larva ikan nila (*Oreochromis niloticus*) tidak dipengaruhi oleh waktu perendaman.

Kata kunci : Gonad Bulu Babi, Sex Reversal, Ikan nila

INTRODUCTION

One type of fish that is relatively easy to cultivate is tilapia (Oreochromis niloticus) because it is very resistant to changing water quality. This fish is often found in places where other types of fish cannot live. When paired with female tilapia, male tilapia grow faster. This is because energy allocation for reproduction is greater than growth, which is a significant economic value of this freshwater fishery commodity (Dagne et al., 2013; Srisakultiew et al., 2013). As a result, the sizes of tilapia fish produced from cultivation vary greatly, resulting in less than ideal harvest weights. Monosexual or single sex culture can overcome this problem (Angienda et al., 2010).

One marine fishery commodity with high potential and economic value is sea urchin, also known as sea urchin. Sea urchin steroid hormone is one of the bioactive substances in sea urchin gonads. This hormone is absorbed by the body more easily and does not cause side effects. One of the most important types of testosterone hormone for the masculinization of animals and fish belonging to the Crustacea is this compound (Susanto et al, 2021; Susanto et al, 2023). Sea urchins, like sand sea cucumbers and other members of the Echinoderms, contain high levels of steroids. In addition, they include marine biota with low fat and high protein content (Susanto et al., 2023).

An alternative natural material that is safer to use is sea urchin. Sea urchin shells, spines and gonad extracts contain active compounds from the alkaloids, steroids, triterpenoids, flavonoids and saponins, based on research by Akerina et al. (2015) and Susanto et al. (2021). Physiologically, there are three sexual steroid hormones, namely progesterone, estrogen and testosterone. Testosterone is a sexual hormone produced in males (Ibrahim, 2001). The hypothalamus can be permanently affected by steroid administration during an important stage of gonad development, namely during the formation of the male phenotype (Susanto et al., 2023).

This research was carried out to determine how administering sea urchin gonad extract at a dose of 8 mg/L for 0, 12, 18 and 24 hours affects the growth of male tilapia fish. It is hoped that this research will produce data on male genital direction (masculinization) and the use of sea urchin gonad extract in the development of tilapia cultivation.

METHODS

The research was carried out from October to December 2022. Maintenance and observations are carried out at the Molecular Biology Research Laboratory, Integrated MIPA Building, Faculty of Mathematics and Natural Sciences, University of Lampung. The production of sea urchin gonad extract was carried out at the Integrated Laboratory of the Center for Innovation and Technology (LTSIT) at the University of Lampung.

Tools and Materials

In this research, the equipment used included a plastic tub with a capacity of 8 liters for acclimatization, a glass aquarium with a volume of 5 liters for care and maintenance, a 1.5 meter long waste suction hose, a petri dish and loop for observing the morphology of tilapia larvae, and a ruler for measure its body length. Measurement of the degree of acidity with a pH meter, water temperature measurement, and DO meter. Making sea urchin gonad extract uses several equipment including a rotary vacuum evaporator, shaker, 500 milliliter measuring flask for making stock solution of sea urchin extract, 250 milliliter baker's glass, test tube, and dropper pipette.

Apart from that, some of the materials used include tilapia larvae that are more than two weeks old, sea urchin gonad extract, pellet feed for tilapia larvae such as PSP, PF 100, and PF 500, water (for rearing media), filter paper, and methanol.

Experimental Design Method

The Completely Randomized Design (CRD) method was the method used in this research. There were 12 experimental units with 3 repetitions of each treatment. The treatment design used is as follows:

SRA : Control
SRB : Soaking in sea urchin gonad steroid extract 8 mg/L for a period of time time 12 hours
SRC : Soaking in sea urchin gonad steroid extract 8 mg/L for a period of time time 18 hours.
SRD : Soaking in sea urchin gonad steroid extract 8 mg/L for a period of time time 24 hours.
(Susanto *et al,* 2018)

Procedures

The research began with preparing tilapia larvae aged 13-14 days, and preparing tanks for treatment and rearing.

1. The sea urchin gonads were removed, separated from the shell, and stored in a freezer at 40 degrees Celsius. In methanol, sea urchin gonad extract was macerated with a ratio of ingredients and solvent of 1:3 (weight/volume), and shaken for 72 hours with a shaker speed of 180 rpm. Followed by filtering the extract with Whatman number 1 filter paper and then evaporating it with a rotational vacuum evaporator at a temperature of 37 to 400 degrees Celsius. After that, the extract is stored at a cooling temperature (0-4 degrees Celsius) and is ready for use.
2. Identifying tilapia larvae based on their morphological body characteristics, such as length, completeness of organs, color and age. Also, observe movement and movement activities.
3. Treatment was carried out by immersing at different time intervals (0, 12, 18, and 24 hours) in sea urchin gonad extract at a dose of 8 mg/L.
4. Maintenance. After the tilapia larvae were soaked in the treatment tank, they were transferred to the rearing tank at a density of 20 fish per aquarium. During maintenance for a period of 60 days, the pellets become fish food which is given every morning and evening. Efforts to maintain water quality are carried out by siphoning and changing the water every three days, and temperature, DO and pH measurements are carried out at intervals of every ten days.

Data retrieval

1. The formula for calculating the percentage of gender formation is::

$$J\ (\%) = \frac{\text{Number of male fish}}{\text{number of fish samples}} x100\% \quad (1)$$

$$B\ (\%) = \frac{\text{Number of female fish}}{\text{number of fish samples}} x100\% \quad (2)$$

$$I\ (\%) = \frac{\text{Number of fish not identified}}{\text{number of fish samples}} x100\% \quad (3)$$

2. Survival Rate
   Effendi (1997) stated that the survival value of tilapia fish can be calculated by applying a formula:

$$SR = \frac{Nt}{No} x100\% \quad (4)$$

Information:
SR = survival of test animals
Nt = number of individuals at the end of the study
No = number of individuals at the start of the study

Data analysis

Analysis of variance (One Way ANOVA) is used to process data in the SPSS 16 program. The BNT (Least Significant Difference) test with a level of 5% ($\alpha = 0.05$) is used if the variance data shows treatments that display significant differences or very different effects real.

RESULT

Formation of Male Tilapia Larvae

The formation of the male sexual phenotype of tilapia (Oreochromis niloticus) larvae, which were 13-14 days old, was observed when given sea urchin (Diadema setosum) gonad extract at a dose of 8 mg/L for 60 days of rearing. The results of these observations are depicted in Figure 1.

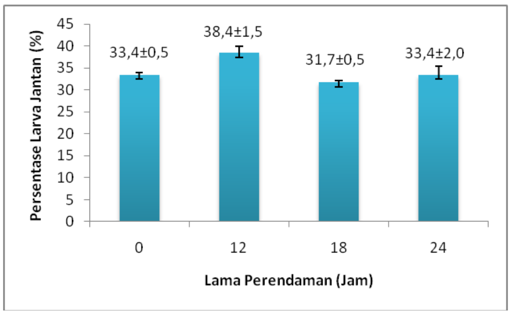

Fig 1. Formation of Male Tilapia Larvae

Based on Figure 1, the results of analysis of variance (One way Anova) show that soaking tilapia fish for a long time in sea urchin gonad solution at a dose of 8 mg/L has no impact on the formation of male genitalia ($p>0.05$). The highest male phenotype occurred at 12 hours of immersion at 38.4%, and the lowest occurred at 18 hours of immersion and controls at 31.7%.

Formation of Female Tilapia Larvae

Figure 2 below shows the results of observations on the formation of the phenotype of female tilapia larvae.

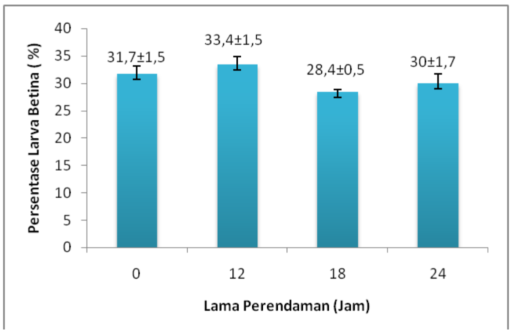

Fig 2. Formation of Female Tilapia Larvae

Long-term soaking in sea urchin gonad extract at 8 mg/L had no impact on female tilapia female genital formation ($p>0.05$), as shown in Figure 2.

The highest female phenotype formation was found at 12 hours of immersion at 33.4%, and the lowest at 18 hours of immersion at 28.4%.

Tilapia Larvae Unidentified

Figure 3 shows the number of tilapia larvae that were not identified during immersion in sea urchin gonad extract.

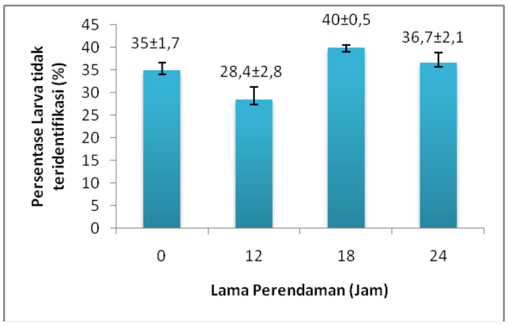

Fig 3. Tilapia Larvae Formation Not Identified

The highest number of unidentified tilapia larvae was 40% at 18 hours of soaking, and the lowest was 28.4% at 12 hours of soaking.

Survival Rate

Figure 4 shows the survival of tilapia larvae (Oreochromis niloticus) within 60 days of rearing.

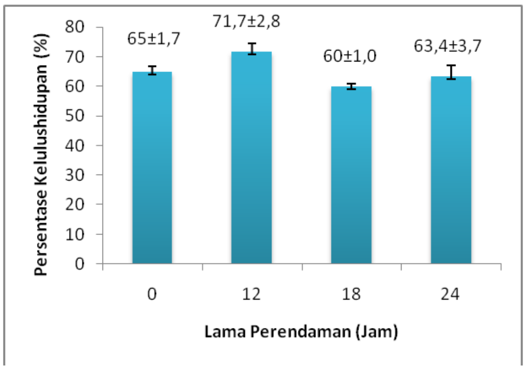

Fig 4. Life cycle of Tilapia Larvae

The results of analysis of variance (one way Anova) showed that soaking sea urchin gonad extract at 8 mg/L during the soaking period had no impact on the survival of tilapia larvae. The highest survival rate of tilapia larvae was 71.7% at 12 hours of immersion and the lowest was 60% at 18 hours of immersion, each with a significance value of $p>0.05$.

Water Quality

To maintain tilapia larvae (Oreochromis niloticus), one of the factors that must be considered is water quality. In this study, water quality was measured by measuring temperature, degree of acidity (pH), and dissolved oxygen (DO) content every ten days for 60 days of maintenance.

Water quality is considered good if it meets the biological needs of fish or is below the acceptable limit for fish to survive (Siregar et al., 2019). Table 1 shows the results of water quality measurements during maintenance.

Table 1. Water Quality for 60 Days of Maintenance

Parameter	Kisaran Nilai	Kisaran Toleransi Menurut Sumber
pH (Unit)	6,59-6,91	6,8-7[1)]
Suhu (^{0}C)	25,4 -27,66	25-30[2)]
DO (mg/L)	3,71 - 3,84	3.6-4[3)]

Source: [1]). Cholik *et al.,* (2005) [2]). Biokani *et al.,* (2014) [3]). Lubis *et al.,*(2017)

Water quality parameters measured every 10 days during 60 days of maintenance experienced increases and decreases. Water pH ranges from 6.76-6.83, while water temperature ranges from 25.56-26.940C, and DO ranges from 3.73-3.80 mg/L.

DISCUSSION

1. Successful Sex Formation

Sea urchins are Avetebrates which are highly nutritious including 18.46% protein, 2.30% carbohydrates, 9.02% fat, 69.47% water content, 0.75% ash and 164.22 kcal energy (Silaban & Srimariana, 2013). However, the composition differs based on type, age, size and living environment (Silaban & Srimariana, 2013). Referring to observations carried out by Akerina et al. (2015), the part of the sea urchin with the highest yield is the gonad (7.10%) and the lowest yield is the spines (0.90%).

According to Akerina et al. (2015) and Susanto et al. (2021), alkaloids, flavonoids, phenol-hydroquinone, steroids, triterpenoids, tannins and saponins are the bioactive parts of sea urchin gonads. Androgenic steroid hormones control the expression of the male phenotype, so that stimulation of this hormone can make fish larvae become males (Ruey-Sheng et al., 2009).

The immersion method was used to direct male tilapia fish in this study. This method was chosen because hormones can enter the body more quickly through the fish's circulation and osmoregulation systems (Saputra et al., 2018). Treatment was given to tilapia larvae aged 13–14 days. Fish fry begin to undergo the process of sexual differentiation at the age of 7 days after hatching, but their sexual organs have not yet formed definitively (Ariyanto et al., 2010). There are two genetic factors that influence the direction of sexual differentiation, namely the hormonal (endocrine) system and the action of genes on chromosomes and autosomes (Ariyanto et al., 2010). However, the physico-chemical conditions of fish rearing media during the period of sexual lability are part of environmental influences (Devlin & Nagahama, 2002).

The results of the variance test (One Way Anova) showed that a dose of sea urchin gonad extract of 8 mg/L provided the best male tilapia larvae formation of 38.4% during 12 hours of immersion in the solution.

Raising tilapia at low temperatures during the sexual differentiation stage tends to produce more female fish (Phelps and Popma, 2000). Changing temperatures also tend to produce more female fish (Phelps and Popma, 2000). The influence of the gonads on the formation of the genitals decreases (Susanto et al., 2018).

Reversal sex of tilapia at temperatures below 240 degrees Celsius will reduce the success of male individual formation and reduce growth rate (Phelps & Popma, 2000). The number of O. mossambicus females is greater at low temperatures (23–250 °C) than at higher and more stable temperatures (Phelps and Popma, 2000). It is thought that one of the factors contributing to a decrease in the rate of male individual formation is a decrease in the temperature of the rearing medium and its temperature fluctuations.

2. Survival Rate

With the highest yield value, sea urchin gonad ethyl acetate extract contains alkaloid compounds, steroid saponins and triterpenoids. Saponin compounds have been proven to have antibacterial, antifungal, antitumor and antihypercholesterolemia properties (Karmiah et al., 2019).

Saponins are glycosides with steroid and triterpenoid aglycones. Saponin can form foam when shaken with water because its polar groups (sugar) and non-polar groups (non-sugar) have active properties. Steroid saponins are often found in monocotyledonous plants, nuts and a number of marine biota because they are fat soluble (Soleimani et al., 2017).

According to Kristianti et al. (2008), the active components of fat-soluble terpenoids are steroids/triterpenoids and saponins. This component has the ability to penetrate bacterial cell walls by stopping protein synthesis, which changes the constituent parts of bacterial cells (Rosyidah et al., 2010). The bioactive components in a material influence its anti-bacterial activity. The types of bioactives that function to inhibit bacterial activity usually consist of saponins, terpenoids, steroids, alkaloids and flavonoids (Darsana et al., 2012). This supports previous research findings that saponin, steroid and triterpenoid groups of compounds are the source of bioactive compounds found in sea urchin gonad extract (Susanto et al., 2021).

The results of analysis of variance (One way Anova) showed that the survival of tilapia larvae (Oreochromis niloticus) was not influenced by the length of soaking in sea urchin gonad extract solution. Biotic (population density, age, and adaptability) and abiotic (environment) are two components that can influence fish survival rates (Karimah et al., 2018).

Soaking in sea urchin gonad extract for twelve hours resulted in the highest viability of 71.7%. This may be because fish larvae are still sensitive to changes in temperature and rearing conditions. Organisms in water use temperature to do many things. According to Effendi (2003), drastic temperature changes can disrupt the life of organisms and cause death due to increased toxicity of dissolved contaminants and decreased dissolved oxygen in water.

Death of tilapia larvae occurred on the second to eighteenth day after treatment and the thirtieth day. After that no more dead fish were found. The cause of fish death occurs due to stress due to transfer from the treatment tank to the rearing tank and the conditions of the rearing environment.

3. Water Quality

For 60 days, the temperature of the maintenance medium ranged between 25.56 and

26.940 Celsius. This temperature is relatively stable during both care and maintenance because the research was carried out in a closed room and was not affected by outside temperature. A water pH between 5-11 is a pH that tilapia can tolerate. In waters with neutral acidity or low alkanity, tilapia can grow and develop well (Indriati & Hafiludin, 2022). Fish pH that is not ideal can cause stress, disease, decreased productivity and low growth. In most cases, the pH of the water during the day is higher than in the morning. This is caused by photosynthesis of natural food, including phytoplankton, which absorbs carbon dioxide (Supriatna et al., 2020).

According to Effendi (2003), sunlight will be absorbed and converted into heat. The water temperature of the rearing medium changes in the morning, afternoon and evening because the rearing container is exposed to direct sunlight. Fish can die due to stress caused by excessive temperature fluctuations (Pratama 2009). During 60 days of maintenance, the dissolved oxygen (DO) content was 3.71-3.84 mg/L, which was considered good because Lubis et al. (2017) noted that the dissolved oxygen range of 3.6-4 mg/L is a level that can be tolerated in fish farming.

CONCLUSSION

Based on the results of this research, it can be concluded that, although variations in soaking time do not have an impact on the formation of male tilapia larvae, soaking for 12 hours gives better male tilapia results than soaking for 0, 18, 24 hours.

REFERENCES

Akerina, F.O., Nurhayati, T. & Suwandy, R. (2015). Isolasi dan karakterisasi senyawa antibakteri dari bulu babi. *JPHPI* 18(1), 61-73.

Angienda PO, Aketch BO, Waindi EN. 2010. Development of All-Male Fingerlings by Heat Treatment and The Genetic Mechanism of Heat Induced Sex Determination in Nile Tilapia Oreochromis Niloticus. *International Journal of Biological and Life Sciences.* 6: 38-43

Arikunto dan Suharsimi. 2019. *Prosedur Penelitian Suatu Pendekatan Praktik.* PT Rineka Cipta. Jakarta.

Ariyanto Didik, Komar Sumantadinata, dan Agus Oman Sudrajat. (2010). Diferensiasi kelamin tiga genotipe ikan nila yang diberi bahan aromatase inhibitor. *J. Ris. Akuakultur.* 5(2):165-174.

Aprillia, H.A., Pringgenies, D. & Yudiati, E. (2012). Uji Toksisitas Ekstrak Kloroform Cangkang dan Duri Landak Laut (*Diadema setosum*) Terhadap Mortalitas Nauplius artemia sp. *Journal of Marine Research* 1(1), 75-83.

Benfey, T.J., 2001. Use of Sterile Triploid Atlantic Salmon (*Salmo Salar L*,) for Aquaculture in New Brunswick, Canada. *ICES Journal odf Marine Science*, 58: 525-529.

Biokani,S.,Jamili, S. Dan Sarkhosh J.,2014.The Study of Different Foods on Spawning Efficiency Of Siamase Figting Fish (spesies : *Betta Splendens*, family :*Belontiidae*). *Marine Science*. 4(2) : 33-37.

Cholik, F., Jagatraya, A.G., Poernomo, R.P., dan Jauzi, A. 2005. *Akuakultur Masyarakat Perikanan Nusantara (MPN) dan Taman Kuarium Air Tawar*. Jakarta. Kualitas Ikan Nila. Graha Ilmu: Jakarta.

Dagne A, Degefu F, Lakew A 2013 Comparative Growth Performance of Mono-sex and Mixed-sex Nile Tilapia *Orreochromis niloticus* l. In Pond Culture System at Sabeta, Ethiopian. *Internasional Journal Of Aquaculture* 3(7): 30-34.

Darsana IGO, Besung INK, Mahatmi H. 2012. Potensi Daun Binahong (*Anredera cordifolia (Tenore) Steenis*) dalam Menghambat Pertumbuhan Bakteri Escherichia Coli secara In vitro. *Indonesia Medicus Veterinus* 1(3):337-351

Devlin, R.H. & Nagahama, Y. 2002. Sex Determination And Sex Differentiation In Fish: An

Overview Of Genetic, Physiological, and Environmetal Influences. *Aquaculture*, 208: 191-364.

Effendi, Hefni. 2003. *Telaah Kualitas Air* : Bagi Pengelolaan Sumber Daya dan Lingkungan Perairan. Penerbit : Kanisius. Yogyakarta

Handayani, N., G. Nugroho,S., Sri, M. 2012. Pengaruh Ekstrak Steroid Teripang (*Holoturia scabra* Jaeger) dengan Lama Perendaman yang Berbeda Terhadap Maskulinisasi Juvenil Lobster Air Tawar (*Cherax quadricarinatus*). *Prosiding Seminar Nasioal Sain, Matematika Informatika, dan Aplikasinya,* Lampung. Hal. 215-219.

Ibrahim M. 2001. *Isolasi Dan Uji Aktivitas Biologi Senyawa Steroid Dari Limbah Laut (Discodoris sp).* [tesis]. Pascasarjana Institut Petanian Bogor. Bogor.

Indriati P.A, Hafiludin. 2022. Manajemen Kualitas Air Pada Pembenihan Ikan Nila (*Oreochromis niloticus*) di Balai Benih Ikan Teja Timur Pamekasan Management Of Water Quality For Tilapia (*Oreochromis niloticus*) Hatching Fish At Balai Benih Ikan Teja Timur Pamekasan. *Juvenil*. 3(2) : 27-31.

Karimah Ulfatul, , Istyanto Samidjan , Pinandoyo. 2018. Performa Pertumbuhan dan Kelulushidupan Ikan Nila Gift (Oreochromis Niloticus) yang Diberi Jumlah Pakan yang Berbeda. *Journal of Aquaculture Management and Technology*. 7(1) : 128—135

Karmiah, Reymon, Muhammad Azdar Setiawan, Evi Apriani Arifin, Musdalipah. 2019. Identifikasi Senyawa Saponin Ekstrak Etil Asetat Gonad Landak Laut (Diadema Setosum) dan Efektivitas Antihiperkolesterol terhadap Mencit balb/C Hiperkolesterolemia. *JURNAL MEDIKA UDAYANA*. 18(2).

Kristianti, A. N, Amina, N.S, Tanjung, M., Kurniadi, B. 2008. *Fitokimia*. Universitas Airlangga. Surabaya.

Lubis, M.A, Muslim, Mirna, F.2017. Maskulinisasi Ikan Cupang (*Betta Sp.*) menggunakan Madu Alami melalui Metode Perendaman dengan Konsentrasi Berbeda. *Jurnal Akuakultur Rawa Indonesia.* 5(1):97-108.

Phelps, RP., Popma, T.J., 2000. Sex Reversal of Tilapia. Page 34-59 in B.A.Costa-Pierce and J.E. Rakocy. (Eds.) Tilapia Aquaculture in the Americas, Vol 2. *The Word Aquaculture Society*, Baton Rounge, Louisiana, United States.

Pratama, 2009. *Morfologi Ikan Nila*. Airlangga. Jakarta. PT Agromedia Pustaka. Jakarta Selatan.

Rosyidah. K., Nurmuhaimina. S.A, Komari. N, Astuti. M.D. 2010. Aktivitas Antibakteri Fraksi Saponin dari Kulit Batang Tumbuhan Kasturi (*Magnifera casturi*). *Alchemy.* 1(2):53-103

Ruey-Sheng, W., S. Yeh., T. Chii-Ruey. and C. Chang. 2009. Androgen Receptor Roles in Spermatogenesis and Fertility: Lessons from Testicular Cell-Specific Androgen Receptor Knockout Mice. Endocr Rev., 30(2): 119-132.

Santoso, B. (1996). *Budidaya Ikan Nila*. Kanisius. Yogyakarta.

Saputra Anugrah, Astri Wulandari, Ernawati, Muhammad Amri Yusuf, Irvan Eriswandy, Andi Aliah Hidayani. 2018. Penjantanan Ikan Gapi, Poecilia Reticulata Peters, 1859 dengan Pemberian Ekstrak Jeroan Teripang Pasir (*Holothuria scabra*). *Jurnal Iktiologi Indonesia. 18(2):127-137.*

Silaban B dan Srimariana E. 2013. Kandungan Nutrisi dan Pemanfaatan Gonad Bulu Babi (*Echinotrixs Calamaris*) dalam Pembuatan Kue Bluder. *Jurnal Pengolahan Hasil Perikanan Indonesia* 6 (2):108-118.

Siregar, S., Mochamad, S, Marini, W. 2018. Maskulinisasi ikan cupang (*Betta spelendens*) Menggunakan Madu Alami Melalui Metode Perendaman. *Jurnal Akuakultur Rawa Indonesia*. 6(2) : 141- 152.

Srisakultiew, P., Kamonrat, W. 2013. Immersion of 17α-methyltestosterone Dose & Duration on Tilapia Masculinization. *Journal of Fisheries Sciences* 7(4): 302-308.

Soleimani, Soolmaz, Morteza, Y., Soheila, M., Hassan, R., Nargers, A.B. 2017. Identification

And Antioxidant Of Polyhydroxylated Naphthoquinone Pigments from Sea Urchin Pigments of Echinometra mathaei. *Medical Chemistry* 10..1007/s0004-016-1586-y.

Susanto, G.N., Sutyarso, H Busman, N R Kurniawan and S M Hasanah. 2021. Genital Reversal Of Betta Fish By Immersion Using Steroid Extract Of Sea Urchins. *Proceedings of IOP Conference Series : Earth an Environmental Science* 674 012057.

Susanto, G.N., Widiastuti, E.L., Rustanti, T., Hadi, S. 2023. Immersion In Sea Cucumber's Steroid Extract to Increase Male Production of Juvenile Freshwater Crayfish. *Fish Aquat Sci*. 26(1):48-57.

Susanto, G. N., Supono, Ikrom, F. D. 2018. Sex Reversal of Juvenile Freshwater Crayfish (Cherax quadricarinatus) Influenced by Steroid Extract of Sea Cucumber and 17α-Methyltestosterone Hormone at Different Temperatures. *ANP Conference Proceedings* 2002,020027.