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**THE EFFECT OF 17 α -METHYLTESTOSTERONE HORMONE APPLICATION
ON MASCULINIZATION AND GROWTH RATE OF VANAME SHRIMP LARVAE
(LITOPENAEUS VANNAMEI, BOONE 1931)**

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ABSTRACT

Sustainable development of *Vaname* shrimp aquaculture in the future depends on several things, one of which is the availability of the male parent in a sufficient quantity. Regarding this problem, sex reversal can be performed as the solution. Sex reversal is a technique of sex genetic differentiation to change sexes artificially from males genetically into phenotype females or vice versa. This research was intended to obtain the maximal mono-sex (single sex) male of *Vaname* shrimp using 17 α -methyltestosterone (MT) hormone through sex reversal technology to the limited sex change in *Vaname* shrimp, in which to date the *Vaname* male parent gained is still dependent on natural selection. This research used a descriptive and experimental method. The experimental research design used was a completely randomized design (RAL – *Rancangan Acak Lengkap*). The treatment given was immersing the shrimp larvae in 17 α -methyltestosterone (MT) for 8 hours at different doses of 0 mg/L, 5 mg/L, 8 mg/L, and 12 mg/L. The immersion of the post-larvae (PL1) of *Vaname* shrimp using the 17 α -methyltestosterone hormone gave a significant result ($P < 0.05$) on all of the treatments and control in which the highest percentage of male *Vaname* shrimp was obtained from the treatments with 8 mg/L and 12 mg/L doses, respectively reaching 64% and 66.7%. As for the weight growth, the immersion of the post-larvae (LP1) growth of *Vaname* shrimp using 17 α -methyltestosterone hormone provided a very significant result ($P < 0.05$) on all the treatments and control in which the highest weight growth (weight gain) and *Specific Growth Rate of Body Weight* (SGR BW) were shown by the treatments with 8 mg/L and 12 mg/L doses, respectively reaching 1.5415 grams and 1.5871 grams.

KEY WORDS

17 α -methyltestosterone, masculinization, *Litopenaeus vannamei*, post-larvae.

Shrimp is one of the export commodities with a high economic value and great demand by the world market. Production of shrimp, especially Vaname shrimp, is always increasing every year, especially Vaname shrimp. Vaname shrimp is an introduced shrimp originating from the western coast of Latin America and has been successfully cultivated in Indonesia. Production of *Vaname* shrimp during 2010-2014 increased by 20.49% from 206.578 tons to 411.729 tons (the Ministry of Maritime Affairs and Fisheries, 2014).

Fishery aquaculture activities, especially shrimp farming, continue to be developed in Indonesia due to the increased consumer demand from time to time, that is to meet the needs of export markets. The world market's high demand in this commodity is a potential opportunity for Indonesia's natural resources to increase the country's foreign exchange value from the aquaculture sector.

The sustainable development of shrimp aquaculture in the future highly depends on several things, including the availability of male *Vaname* shrimp in a sufficient quantity. So far, male *Vaname* shrimp is still dependent on natural selection, causing the existence of *Vaname* male parent is on a limited scale. In order that *Vaname* parent still continues to exist

in the future, a breeding program needs to carry out. Such a program is hoped to make the availability of *Vaname* male parent no longer depend on natural selection. In line with this, sex reversal can be one means that provides a solution to the problem. Sex reversal is a technique of sex genetic differentiation to change sexes artificially from males genetically into phenotype females or vice versa. Changes in sex can occur naturally and artificially.

Physiologically, fish sexes can be directed or determined by using steroid hormones. In a simple, hormone administration aims to influence the balance of hormones in the blood that, at the time of sex differentiation, is very determining whether a particular individual will be male or female, in which the hormone is injected from outside the body Sumantadinata and Carman (1995). This research aimed to obtain the maximal mono-sex (single sex) male of *Vaname* shrimp using 17 α -methyltestosterone (MT) hormone through post-larvae immersion – sex reversal technology to the limited sex change in *Vaname* shrimp, in which to date the *Vaname* male parent gained is still dependent on the natural selection. Therefore, as an alternative to solve the problem, it is necessary to conduct relevant research soon so that the *Vaname* male population no longer depends on nature.

MATERIALS AND METHODS OF RESEARCH

Research Material. The tools used in this research were concrete tubs, aerator, hoses and aeration stones, camera, duster, tissue, electric microscope, petri dish, a digital scale, caliper, measuring cups, refrigerator, basins/ washbowls, thermometer, DO meter, pH meter, and refractometer.

The materials used in this research were post-larvae of *Vaname* shrimp (*Litopenaus vannamei*), post-larva feed, Methyltestosterone hormone, ethanol of 70%, formalin of 10%, Aquadest, hematoxylin and label paper.

Test Animal Preparation. The test animals used in this research were 1-day old post-*Vaname* shrimp larvae (*Litopenaus vannamei*) originating from the Production Center of Parent of Superior Shrimp and Mollusk (*Balai Produksi Induk Udang Unggul dan Kekerangan – BPIU2K*), Karangasem, Bali. The post-larva selection was done morphologically based on its morphological features such as length, weight, organ completeness, and body color. The acclimatization did not require a long time because the *Vaname* shrimp examined came from the same place, namely *BPIU2K*.

Test Animal Immersion into 17 α -methyltestosterone hormone solution. The post-larvae (PL) of *Vaname* shrimp were randomly selected and stocked into immersion aquariums with a density of 10 PL/L, so the total post-larvae stocked in each aquarium was 100 post-larvae (PL). The immersion process was performed for 8 hours at doses of 0 mg/L, 4 mg/L, 8 mg/L, and 12 mg/L with three replications. After that, the post-larvae were filtered and re-stocked in an aquarium containing 10 liters of new seawater. The control treatment was prepared without the addition of 17 α -methyl testosterone hormone.

Test Animal Rearing. The rearing of the post-*Vaname* shrimp larvae (*Litopenaus vannamei*) was carried out in 12 units of aquarium container, each of which contained 10 liters of water with a density of 10 PL/ liter, so the total post-larvae stocked in each aquarium amounted to 100 post-larvae (PL). The rearing was done indoors to make the change between night and day not too significant so that the post-larvae were not stressed by temperature changes. Substitution of water as the maintenance media was done every two days as much as 60-70% by being siphoned using a small hose and then replaced with clean and sterilized fresh water. At the end of the rearing period, namely PL60, the post-larvae in each aquarium were collected by means of siphoning the water first, and then the collected post-larvae from each aquarium were weighed for the purpose of growth rate analysis.

Determination of sex and sex ratio. Observation of the post-*Vaname* shrimp larvae given 17 α -methyltestosterone hormone treatments through immersion was done at the end of the research. The female post-larvae were characterized by a thelycum which is described as a small indentation between the fourth and fifth walking legs on both sides. Meanwhile, the male post-larvae were characterized by the appendix masculine on a pair of the second natatorial (swimming) legs and the gonophores complex on the fifth walking legs.

Percentage of Weight Growth. At the end of the test, each individual of the post-larvae from each group treatment was weighed and measured to determine the body weight. From the body weight measurement, the average of the final body weight (BW) and specific growth rate (SGR) were calculated. The body weight was measured using a digital scale with a precision of 0.0001 gram.

Statistical Analysis. One analysis of variance direction (ANOVA) was used to determine the difference between the treatments. Duncan test was also conducted to determine the significance of the difference between the treatments. The significance level of the results was set at $P < 0.05$. All the statistics were performed using SPSS (version 16).

RESULTS AND DISCUSSION

Percentage of Male Vaname Shrimp (*Litopenaeus vannamei*). In this research, the identification of male *Vaname* shrimp was performed after 60 days of rearing, in which the post-*Vaname* shrimp larvae were randomly selected and stocked into immersion aquariums with a density of 10 PL/ liter, so the total post-larvae stocked in each aquarium was 100 PL. The immersion process was performed for 8 hours at doses of 0 mg/L, 4 mg/L, 8 mg/L, and 12 mg/L with three replications. Furthermore, the rearing was done for 60 days to prevent the identification from the occurrence of errors. To determine the percentage of male post-*Vaname* shrimp larvae, the external sex was identified using a microscope.

Based on the observed percentage of male *Vaname* shrimp, the treatments of 17 α -methyltestosterone hormone administration with different doses resulted in a higher percentage of male *Vaname* shrimp compared with that without the 17 α -methyltestosterone hormone administration or the control. The observation data on the percentage of male *Vaname* shrimp at the end of this research can be seen in the following table and figure.

Table 1 – Ratio Percentage of Male Vaname Shrimp (*Litopenaeus vannamei*) on Various Treatments and Control

Immersion Duration	Doses of 17 α -methyltestosterone Hormone			
	0 Mg/L	4 mg/L	8 mg/L	12 mg/L
8 Hours	54.7 $\pm$ 2.1 ^a	61.7 $\pm$ 2.1 ^b	64 $\pm$ 4.0 ^{bc}	66.7 $\pm$ 2.3 ^c

The data shown are the mean $\pm$ standard deviation.

The letters denote Duncan Test difference.

Figure 1 – Graph of Ratio Percentage of Male *Vaname* Shrimp on Various Treatments and Control

Based on the data above, it can be seen that the post-*Vaname* shrimp larvae (PL1) immersion using 17 α -methyltestosterone hormone showed a significant result ($P < 0.05$) on all the treatments and control in which the highest percentage of male *Vaname* shrimp was obtained from the treatments with 8 mg/L and 12 mg/L doses, respectively achieving 64% and 66.7%. The 17 α -methyltestosterone hormone containing steroids is an androgenic hormone, thus causing an increase in testosterone levels in the body of *Vaname* shrimp so as to generate masculinizing effects. According to Donaldson *et al.* (1978), steroids are hormones that can affect the reproduction of animals, stimulate the growth and differentiation

of genitals, as well as affect the behavior of fish and shrimp. Meanwhile, androgen hormones function to determine male sex and behavior, testicular development, sperm ducts and normal cleavage process of spermatogenesis.

Several factors affecting the success of sex reversal are size and age, duration of treatment, environment (Phelps and Popma, 2000), fish species, genetic, hormonal type, hormone dose, treatment time, (Dunham, 2004). Meanwhile, Tourir (1977) stated that sex reversal on *Macrobrachium rosenbergii* will be achieved well if done in the initial stadia before the formation of gonads. According to Charniaux-Cotton (1954), Hasegawa *et al.* (1993), and Tourir (1977), androgen hormones produced by the androgenic gland are responsible for influencing the development of primary and secondary male sexual characteristics in crustaceans.

Weight Growth of Vaname Shrimp. Based on the observed weight growth (weight gain) percentage of post-*Vaname* shrimp larvae, the treatments of the 17 α -methyltestosterone hormone administration with different doses resulted in a higher weight percentage of male *Vaname* shrimp compared to that without the 17 α -methyltestosterone hormone administration or the control. The observation data of the weight growth percentage of male *Vaname* shrimp at the end of this research can be completely seen in the following table and figure.

Table 2 – Ratio Percentage of Weight Growth and Specific Growth Rate of Body Weight (SGR BW) of Vaname Shrimp (*Litopenaeus vannamei*) on Various Treatments and Control

Immersion Duration	Doses of 17 α -methyltestosterone Hormone			
	0 Mg/L	4 mg/L	8 mg/L	12 mg/L
8 Hours	1.3601 $\pm$ 0.0139 ^a	1.4601 $\pm$ 0.0267 ^b	1.5415 $\pm$ 0.0105 ^c	1.5872 $\pm$ 0.0104 ^d

The data shown are the mean $\pm$ standard deviation.

The letters denote Duncan Test difference.

Figure 2 – Graph of Ratio Percentage of *Vaname* Shrimp Weight Growth on Various Treatments and Control

From the table and graph above, it is known that the post-*Vaname* shrimp larvae (PL1) immersion using 17 α -methyltestosterone hormone showed a very significant result ($P < 0.05$) on all the treatments and control in which the highest weight growth (weight gain) and specific growth rate of body weight (SGR BW) were obtained from the treatments with 8 mg/L and 12 mg/L, respectively reaching 1.5415 grams and 1.5871 grams. The increasing weight growth average of *Vaname* shrimp larvae if compared to the control in this research indicates that the 17 α -methyltestosterone hormone administration through immersion has been able to provide an effective effect on the weight growth of *Vaname* shrimp. This is consistent with Piferrer (2001) stating that the treatment of steroid hormones, in addition to affecting genital changes, can also have an impact on growth. According to Anthony *et al.* (1999), steroid hormones are greatly useful in protein synthesis in muscle formation. Meanwhile, Fullerton (1980) explained that, in addition to having androgenic properties, it

turns out that testosterone has anabolic properties, which can stimulate muscle growth. Because the concentration of dissolved synthetic hormones that enter the shrimp body is different, shrimps that absorb hormones with high concentrations grow much faster than other shrimps.

CONCLUSION

Based on the findings of this research, the use of 17 α -methyltestosterone hormone through immersion can increase the male *Vaname* shrimp percentage by 66.7% and the weight growth percentage by 1.5872 grams.

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