THE EFFECTIVENESS OF USING STEROID EXTRACT OF SEACUCUMBER FOR SEX REVERSAL IN JUVENILE REDCLAW CRAYFISH (*Cherax quadricarinatus*)

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The freshwater crayfish, red claw *Cherax quadricarinatus* is commercially cultured in many tropical countries. Males of this species have larger harvest size than females, so it is proposed to culture of all-male population for increasing yields. However this practice has hindrance, since it is difficult to provide monosex seed in high quantity.To overcome this problem, it is important to undergo a monosex (single gender) cultivation. Although sex of *C. quadricarinatus* is determined by genetics, sex reversal from females to males can be triggered by using steroid hormone at their early juvenile stage. The research was conducted to evaluate the effectiveness of sea cucumber's steroid extract for sex reversal from females to males in juvenile crayfish. The research was designed using factorial complete random design. Two different techniques in giving the steroid hormone, which are by immersion and oral. Two groups of 1-2 weeks old juvenile of *C. quadricarinatus* were immersed in water containing various doses of sea cucumber's steroid extract 2 and 4 mg/L respectively, and the other two groups were administered diet containing either extract of sea cucumber or 17α-methyltestoterone (MT) in dose of 50 mg/kg diets. Sex ratio, survival and growth rate were observed at the end of 50 days juveniles rearing in aquaria. Sex differentiation was determined by visual observation. Data was analyzed by Anova and LSD tests. The results showed that administration of steroid extract of seacucumber was significantly increased the male ratio of crayfish. Treatment dose of 2 mg/L during 18 h immersion was effective to increase male sex percentage from 31.03% (control) to 79.86%. On the other hand, 50 mg/kg of steroid extract of sea cucumber in the diet was more effective to increase the male percentage up to 75,16% than MT. Growth rate of juvenile crayfish, included total length, daily weight gain and biomass, were also significantly improved, while survival rate was insignificant. Thus, the administration steroid extract of seacucumber by both immersion and oral are effective to increase male sex percentage of juvenile crayfish.

Keywords: steroid extract, sea cucumber, sex reversal, redclaw crayfish

Introduction

Red claw freshwater crayfish (*Cheraxquadricarinatus*) is a fishery commodity originating from Queensland, Australia and has been introduced to Indonesia since 1991 (Lawrence and Jones 2002 and Edgerton 2005). Crayfish culture business is increasingly favored by farmers because it has a good adaptability to the environment, so it has the potential to be cultivated throughout the year. However, in the enlargement process there are obstacles because the male lobster weighs more heavily than the females after 7-8 months of maintenance (Curtis and Jones, 1995).

As the development of aquaculture one way to increase production is through the cultivation of monosexual sex by directing sex reversal (genital reversal). This engineering technique is widely used in the process of masculinization of fish and some animal crustacean members, such as giant prawns, crabs and freshwater crayfish. Sex reversal is a way of reversing the direction of genital development of animals that should be directed male to female or vice versa. This technique is performed in a labile period before the gonads differentiate and are still sensitive to hormone treatment (Triajie, 2010). Sex reversal only alters phenotype rather than its genotype (Wichins and Lee, 2002). Sex reversal can be done through natural or synthetic hormones, in the form of hormones androgen or estrogen that is not harmful to biota cultivation and consumers, and given according to the desired sex. In fact the steroid hormone widely used in genital reversals is 17α methyltestosterone. The use of this synthetic hormone has a very high rate of 96-100%, but it causes residues that are potentially bad for human health, environment and cultivation organisms (Zairin, 2003), so research needs to be done to obtain a natural steroid source that is safe for humans and environmentally friendly. One way to take advantage of natural hormones extracted from the innards of sea cucumbers (*Holothuriascabra*). Lieberman-Burchard test results and bioassay using chicks showed that the extract of sea cucumber proved to contain steroids. The largest yield obtained from 1 kg of wet viscera (21.28 g of crude extract) contains steroid 6,124 µg / kg testosterone (Riani et al., 2005). According to Kustiariyah (2006) extract of sea cucumber containing testosterone which can be used for genital reversal in fishery commodities, such as giant prawns (Kusmini et al., 2001; Sarida, 2008; Triajie, 2008; Riani et al., 2010), gapi fish Arfah et al., 2005a, b; Riani et al, 2008), where males are more valuable than females.

In addition to the steroid hormone from innards of sea cucumber and 17α metiltestosterone, the process of sex gestation can be triggered through environmental temperature manipulation. Temperature affects the rate of chemical reactions in the body, including the rate of metabolism (El-Fotoh et al., 2014). Increased temperature can speed up the metabolism process so that the lobster appetite also increases. This certainly can help the absorption of steroid hormones contained in the feed so as not damaged and effective entry

into the body. Optimal water temperature will affect the various mechanisms in the body, especially during the period of sexual differentiation is very sensitive to hormonal stimulation and temperature. Conversely, the temperature is too low to cause gonadal sensitivity in the formation of genitals to be slow.

In tilapia the temperature 28 ° C causes male formation of 52,33%, while at 36 ° C able to produce tilapia till 81% (El-Fotoh et al., 2014). In catfish channel fish the influence of temperature 29-30 º C can give effect of forming males equal to 69,5% (Patino et al., 1996). Some of the results of this study indicate that the temperature also affects the process of reversal sex (sex reversal). Therefore, a combination of steroid hormone extracts of sand cucumber and 17α metiltestosterone with different temperature variations on genital variation in juvenile freshwater lobster should be conducted. This research is expected to answer the problem in freshwater crayfish cultivation, especially in the effort to increase production through monosexual cultivation.

Materials and Methods

Animals

Juvenile crayfish (*Cherax quadricarinatus*) were hatched at the aquatic biology research laboratory, Department of Biology, University of Lampung. Juvenile crayfish at an average age of 3-4weeks and a weight of 0.02-0.2 g were then moved in glass aquaria of 60 x 40 x 30 cm containing 20 l of dechlorinated tap water (pH 6.8-7.4), under continuous aeration, temperature of 26–27_C and 12:12 light:darkness photoperiod. They were fed daily ad libitum on pellet. During nursery rearing, juveniles were observed weekly understereoscopic microscope to determine sex, using the presence/absence of the genital open ings at the basis of the third (females), fifth (males) or both (intersex) pair of walking legs. When the juveniles could be sexed, 20 early juveniles were selected and randomly assigned to each of the following experimental group

Morphological observations

The following morphological variables were recorded at the time of harvest (age 2 months) for each male individual (n = 20, for random control samples of males/females (n = 10 of each sex) from the same population, and for the 2-month-old individuals: body weight (+/- 0.1 g), carapace length, total body length (+/- 0.1 mm). Along with the sexual differentiation in juvenile *C. quadricarinatus*, changes in the morphology of the pleopod was observed carefully.

Animal Maintenance Test and Treatment:

Juvenile freshwater crayfish used ± 1-2 weeks or 2-2.5 cm long body length of ± 300 heads from Gemma Farm cultivators in Klaten, Central Java. Juvenile lobsters morphologically have perfect organs, in good health and taken from a well-selected parent. Before putting in the container, juvenile lobster is first weighed to determine the initial weight, and then acclimated in the laboratory for 2-3 days before being kept in a tub or aquarium.

The feed used is the sinking pellet type from Gemma Farm with 40% protein composition, 12% water, 6% fat and 3% fiber. The hormone used is a type of natural steroids from extracts of sea cucumber innards which extraction process is done in the laboratory of Organic Chemistry, University of Lampung. While the synthetic hormone used type 17α metiltestosteron from Biotech Argo-lab (Sidoarjo). The dose of hormone used is 50 mg / kg of feed.

Pellet feed plus steroid hormone by weighing the hormone extract sea cucumber sand and hormone 17α metiltestosteron each 50 mg. Furthermore, the hormone dissolved in 250 ml of 70% alcohol, put in a sprayer, then sprayed evenly on the feed. After the hormone is sprayed, the feed is then stirred and dried for 1 day until all alcohol evaporates, then stored in a closed container. Feed on the controls do not add steroid hormones, treated only with different temperatures. Hormonal feeding is done for 50 days. Each treatment consisted of three replications. The amount of feed given is 8-10% of the total weight of juvenile lobster. Frequency of feeding as much as 2 times a day that is in morning and evening.

Juvenile freshwater crayfish are preserved for 50 days or until their secondary sex features are clearly visible. The male genital traits are shown in the presence of a bulge at the base of the fifth stalk (periopod) rod calculated from the foot of the path under the mouth called pethasma, whereas the female is shown the existence of a round hole located on the base of the third periopod called thelicum.

To keep the water quality is always optimal for the growth of lobsters, on each container is given enough aeration. Substitution of water is done as much as 30-50% every 3 days by means of basic water menyipon or dirt settling, then added water replacement to the volume of water as before. Water quality parameters measured are temperature, pH, dissolved oxygen content (DO) and hardness. Observations of temperature, DO and water pH were performed every 3 days, while the hardness was measured at the end of the study. The value of water quality range during the study is presented in Table 1.

Table1. Measurement of water quality in the aquaria

Treatment	DO (ppm)	Temperature (° C)	pH	Hardness (ppm)
KT. 27	3,0 – 3,9	26 – 27	7,4 - 7,8	83,00
KT. 31	2,9 – 4,4	30 – 31	7,4 - 7,9	
ST .27	3,2 – 5,3	26 – 27	7,2 - 7,8	
ST .31	3,1 – 4,8	30 – 31	7,4 - 7,9	
MT .27	3,2 – 4,5	26 – 27	7,4 - 7,8	
MT .31	3,1 – 4,1	30 – 31	7,4 - 7,8	

Research Design :

This research was arranged with Factorial Completely Randomized Design Method, consisting of temperature factor of 2 levels (temperature 27 ° C and 31 ° C) and hormone factor 2 level (sand cucumber steroid and 17α metiltestosterone) with each control, 6 treatments and each treatment with three replications. The treatments are as follows:

KT.27: control at 27 ° C

KT.31: control at 31 ° C

ST.27: administration of 50 mg / kg sand cucumber steroid at feed temperature ± 27 ° C

ST.31: provision of 50 mg / kg sand cucumber steroid on feed ± 31 ° C temperature

MT.27: administration of 50 mg / kg of 17α methyltestosterone at feed temperature ± 27 ° C

MT.31: administration of 50 mg / kg of 17α methyltestosterone at feed temperature ± 31 ° C

Parameter of Research:

The formation of individual male sex

The percentage of successful formation of male sex can be calculated by the formula:

Number of male lobster

J (%) = x 100% (Zairin, 2003)

Number of lobster samples

Survival

SR = Nt / No x 100% (Effendie, 2004)

SR = lobster survival test (%)
Nt = number of test lobsters at the end of the study
No = number of test lobsters at the beginning of the study

Growth Rate of Daily Weight

The growth rate of daily weight is calculated using the formula:

$$GR = \frac{Wt - Wo}{t}$$ (Effendie, 2004)

GR = daily growth rate (g / day)
Wt = average weight of lobster on day t (g)
Wo = average weight of lobster on day 0 (g)
t = maintenance time (day)

Biomass

$$\Delta W = Wt - Wo$$ (Effendie, 2004)

W = biomass (gram)
Wt = biomass at the end of the study (gram)
Wo = Biomass at the beginning of the study (gram)

Feed Conversion Rate

$$FCR = \frac{F}{(Wt + D) - Wo}$$

FCR = feed conversion ratio
F = amount of feed given during maintenance (kg)
Wt = lobster weight at end of maintenance (kg)
D = dead lobster weight during maintenance (kg)
Wo = weight at start of maintenance (kg)

Data Analysis

The research data includes the formation of sex, life, daily growth rate, biomass, and feed conversion ratio. The data is then processed by the analysis of variance, if there is a real difference then continued the BNT test (the smallest real difference) using SPSS 19 program.

Results and Discussions

Results

The complete results of the effect of natural steroid hormone extracts of sand cucumber and synthetic hormone 17α methyltestosterone at different temperatures to male formation in freshwater juvenile lobsters can be seen in Table 2

Treatment	Percentage of individuals male (%)	Survival (%)	Daily growth rate of weight (g)	Biomass	Feed Conversion Ratio
KT 27	42,06 ±8,36[a]	23,33±10,41[a]	1,26±0,25[a]	1,56±1,0[a]	2,98±0,51[a]
KT 31	50,07 ±5,51[a]	80,00±15,00[a]	0,86±0,09[a]	6,87±4,90[a]	3,94±1,83[a]
ST 27	75,16±6,01[b]	75,00 ±8,66[a]	1,53±0,02[b]	11,10 ±1,33[b]	3,25 ±0,73[a]
ST 31	62,5±12,5[b]	50,00±10,00[a]	1,45±0,10[b]	11,02±4,06[b]	2,15±0,19[a]
MT 27	73,79±6,18[b]	56,67±10,41[a]	1,00±0,20[a]	6,72±3,44[a]	3,02±0,20[a]
MT 31	53,33±5,77[b]	53,33±5,77[a]	1,15±0,9[a]	8,95±3,97[a]	2,06±0,31[a]

Different letters in the table indicate a difference between treatments (LSD Test)

Table 2.Percentage of individual males, survival, daily weight rate, biomass, and conversion ratio of juvenile freshwater lobster feed treated with steroid extracts of sea cucumber sand and 17α methyltestosterone at different temperatures for 50 days of maintenance

Percentage of Freshwater Crayfish Males

Based on the result of the analysis of variance to the treatment, the highest percentage of juvenile males obtained in the treatment of steroid hormone from sea cucumber was 27 ° C (ST.27) of 75.16%, while the lowest percentage of males in temperature control was 27 ° C (KT.27) by 42.06% (Table 2). The result of analysis of variance with 95% confidence level, showed that giving steroid hormone and different temperature gave a significant influence (P <0,05). The results of the analysis prove that giving steroid hormone and different temperatures give a positive response to increasing the percentage of male freshwater lobster.

Survival

Based on the analysis of the variance of each treatment, it was found that the highest survival rate was found in the temperature control treatment 31 ° C (KT.31) of 80.00%, while the lowest survival rate was in the temperature control treatment 27 ° C (KT.27) amounted to 23.33%. (Table 2). From the results of analysis of variance with 95% confidence level, showed that giving steroid hormone and different temperature did not give significant effect (P> 0,05).

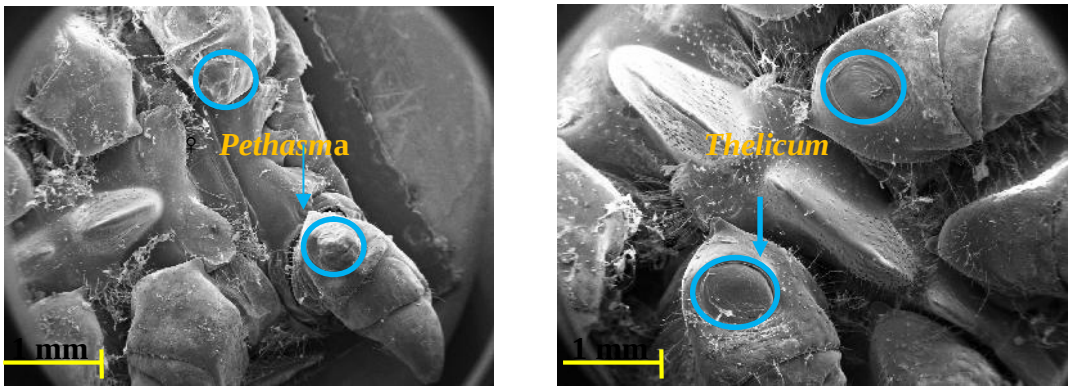

Figure 1. Differences in the formation of sex on male LAT (left) and female (right) has a bulge at the base of the fifth-way foot called pethasma, whereas females there is a round hole at the base of the 3rd way foot called thelicum.

Daily Growth Rate of Weight

Based on the observation for 50 days, the highest daily weight gain of LAT was obtained at the treatment of steroid sand cucumber sand at temperature 27 ° C (ST.27) that is equal to 1.53 gr. While the lowest daily weight gain of LAT was found in the control treatment at 31 ° C (KT.31) of 0.86 gr. (Table 2). From the results of analysis of variance with 95% confidence level, indicating that giving steroid hormone and different temperature give a significant influence (P <0,05).

Biomass

Based on observation on the 50th day, the highest LAT biomass value was obtained in the treatment of steroid sand cucumber sand at temperature 27 ° C (ST.27) of 11,10 gr, while the lowest LAT biomass value at control treatment at 27 ° C (KT .27) of 1.56 grams. (Table 2). From the results of analysis of variance with 95% confidence level, indicating that giving steroid hormone and different temperature give a significant influence (P <0,05).

Feed Conversion

Based on observation on the 50th day the lowest feed conversion value of LAT was obtained at the treatment of 17α methyltestosterone hormone at 31 ° C (MT.31) of 2.06 gr, while the highest LAT feed conversion value at the temperature control treatment was 31 ° C (KT .31) of 3.94 grams (Table 2). From the results of analysis of variance with 95% confidence level, showed that giving steroid hormone and different temperature did not give significant effect (P> 0.05).

Discussion

One of the success factors in the reversal of sex toward the males is the presence of steroid hormones as well as the optimal maintenance environment. In this study the hormone used there are two types of natural hormones derived from sand cucumber extract and synthetic hormone 17α metiltestosterone with a dose of 50 mg / kg of feed. This dose although not 100% effective produces male individuals, but is sufficient to produce the majority of male individuals in juvenile LAT.

In this study the natural hormone used is steroid extract sea cucumber. Sand sea cucumber extract is a hormone that provides the effect of masculinity, because it can increase the amount of testosterone in the body LAT. This is like Triajie (2008) research on juvenile males of prawns, where the highest yield of testosterone hemolymph is found at a dose of 25 mg / l of sand cucumber steroid extract.

Another androgen hormone used in this study was 17α metiltestosterone and gave the male percentage of 73.79%, lower than the use of sand cucumber steroids. These results are different from those of Carman et al. (2008) with 17α metiltestosterone dose 50 mg / kg resulting in a smaller percentage of males of 59.96% in the same animal. Different also with research Kusmini et al. (2001) in 20-day prawn larvae with 17α methyltetosterone at 35 mg / kg dose feed for 30 days resulting in 80.91% males.

According to Zairin (2004), doses of hormones that are too low, causing the process of sex reversal lasted less perfect. While the dose is too high, there is a tendency the fish will become sterile. Therefore, appropriate dose conformity is required. In this study, a dose of the hormone 50 mg / kg of feed, simply provide a high percentage of males when compared

with controls. The administration of steroid hormones in small amounts given to individuals who have not developed gonads yet, will directly affect the hypothlamus regularly during the critical stages of gonadal development and character formation of males. Suspected testosterone affects neurons through the preoptic hypothalamus section with synapses secreted in gonadotropin releasing factor (Kusmini, 2001).

The natural hormone of sea cucumber steroids is capable of giving higher percentage of males when compared with the use of hormone synthesis 17αmetiltetosteron. This can answer the problem in the cultivation, which is the hormone synthesis, residual properties that have a negative impact on human health. Cucumber steroid hormone can be an alternative to replace the hormone synthesis 17αMetiltetosteron.

In addition to hormonal influences, the right environmental temperature can affect the process of forming individual males in LAT. LAT is a poikiloterm animal whose body temperature rises and falls according to ambient temperature (Brotowidjoyo et al, 1995), therefore all physiological processes of LAT are influenced by ambient temperature (Efendi, 2003). Water temperature affects the behavioral response, metabolic and reproductive processes (Efendi, 2003).

In this study in addition to the use of hormones are also given different temperature treatment that is 27 ° C and 31 ° C. At ST treatment 27 were given steroid carcass of sand cucumber at 27 ° C and MT.27 was given 17α methyltetosterone at 27 ° C giving male percentage 75,16% and 73,79% respectively. This result is similar to that of Arfah et al. (2005) at a temperature of 27 º C which resulted in the highest percentage of male gappy male sex of 92.7%. In the process of sex reversal, environmental factors are made in such a way that it can affect the transcription and transcription of sex determinant determinants (Sucipto et al., 2004). By making the environment predominant or saturated androgen hormones, the hormonal balance will be disrupted, so the phenotypic sex determination will further lead to the formation of a male individual.

In (Table 2) it is known that the presence of steroid hormones in feed and different temperatures causes the percentage of survival of LAT increasing. From the results of the mean of survival proves the existence of steroid hormone in the diet and the different temperatures have a positive effect on increasing the percentage of survival LAT, although not significant. The treatment of steroid hormone sea cucumber sand and different temperature can give the value of survival equal to 62,5% higher if compared to control which only equal to 51,67%. According to Ridzwan et al. (2003) sea cucumbers have useful bioactive components in the medical and health fields consisting of triterpene glycosides (saponins), chondroitin sulfates, glycosminoglycan, sulfated polysaccharides, sterols (glycosides and sulfates), phenolics, peptides, cerberosides, and lectins. Sea cucumbers have biological and pharmacological activities such as anti-cancer, anti-coagulant, anti-

hypertensive, anti-inflammatory, antioxidant, and anti-tumor (Bordbar et al., 2011). Sea cucumber extract also has antibacterial and anti fungal activity. Nimah et al. (2012) states that the extract of methanol sea cucumber H. scabra and acetyl acetate have inhibitory power against bacteria P. aeruginosa and B. cereus. H. scabra sea cucumber extract has antifungal activity of Candida albicans (Pranoto et al., 2012), in rough extract of sea cucumber sand also can increase stamina of body. From some of the benefits of previous research the sand cucumber steroid hormone is able to improve LAT body health so as to give a positive response to its livelihood.

The giving of sea cucumber extract had a significant effect on the increase of daily weight of LAT. This further reinforces the opinion that the steroid hormone can stimulate metabolic activity, thus stimulating the growth of LAT. Sagi et al. (2002) states that in addition to having androgenic properties, testosterone turned out to have anabolic properties that can spur muscle growth. Sand sea cucumber extract contains androgen hormones just like metiltestosterone (MT), so it has anabolic properties that can stimulate growth (Fulierton, 1980). The same opinion by Handajani (2006) that steroid hormone can affect the growth of both male and female fish.

Figure 13 shows the treatment of sand cucumber steroid hormone at 27 ° C (ST.27) showed a higher biomass value of 11.10 g compared to a temperature control treatment of 27 ° C (KT.27) of 1.56 gram. This shows that the extract of sea cucumber that is given can increase LAT biomass, because the hormone that is mixed in the feed able to cooperate in accelerating growth.

The final weights obtained gave different effects. This is suspected because the hormone steroids increase androgen content, so that animal aggressiveness increases and affects the rate of metabolism in the body. The increased rate of metabolism affects appetite increase and in the end the rate of growth also increases. This is demonstrated also by Handajani (2006) research in which steroid hormones can affect the growth of both male and female fish. However, in the LAT growth there is a difference between male and female individuals who are clearly seen as characteristic (Widha, 2003).

The other factors besides hormones also contain other ingredients contained in the sea cucumber. According to Nurjanah (2008) the content of collagen in the sand cucumber reaches 80%, while the protein content reaches 86.6%. Sea cucumbers also contain many growth factors that can stimulate cell and tissue regeneration (Huberman, 2000).

In this (Table 2), 17α methyltestosterone hormone at 31 ° C (MT.31) was able to produce a lower feed conversion value of 2.06 when compared with the treatment of sterile sand cucumber (ST) and control (KT). It shows the use of the hormone 17α metiltestosterone is still better when compared with other treatments. Provision of hormones 17α metiltestosterone and temperature variations is able to make the conversion value of feed on

LAT to be efficient. The results of Ayuningtyas (2015) with 17α metiltestosterone and temperature in tilapia were able to give feed conversion value of 2.48.

The hormone 17α metiltestosterone in addition to regulate the sexual development of fish also improves digestion, food absorption, feed conversion as well as other physiological processes (Mukti et al., 2002). With the provision of 17α metiltestosterone will stimulate metabolic activity, so that LAT has a high appetite

Conclusions

From the results of this study can be concluded:

1. Giving steroid cocktail sand and 17α metiltestosteron 50 mg / kg feed on sex reversal to male individuals on LAT (Cherax quadricarinatus) has the highest percentage at 27 ° C that is 75,16% and 73,79%.
2. Provision of sand cucumber steroid hormone is more effective in sex reversal to male individuals in LAT (Cherax quadricarinatus) when compared with 17α methyltestosterone at both 27 ° C and 31 ° C ie 75.16% and 62.5%

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