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Crustacean Hyperglycemic Hormone and Gonad Histology of Female Spiny Lobster (*Panulirus homarus*) Inducted by Laserpuncture

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Abstract: Spiny lobster is one of the potential commodities in Indonesia. On the other hand, there is still a problem to fulfill the export-ready stock of spiny lobster. Production engineering can be utilized as one way to overcome the existing problems. Laserpuncture is a new method of production engineering by utilizing low wavelength helium-neon light that can provide biological stimulation to living things resulting in accelerated development. Laserpuncture induction in crustaceans such as lobsters can be done on the eye stalk and ventral part. This study was conducted using completely randomized design method with 4 treatments and 5 repetitions of each treatment. The treatments given are differences in the duration of laserpuncture induction on female spiny lobster including A (2 seconds induction), B (4 seconds induction), C (6 seconds induction), and control (without induction). This study aims to analyze wheter laserpuncture affects Crustacean Hyperglycemic Hormone. CHH and gonad in female spiny lobster. Data analysis in this study was carried out qualitatively which included Crustacean Hyperglycemic Hormone A & B hormone, gonad histology, and survival rate. The results showed that treatment A (2 seconds induction of laserpuncture) on female spinny lobster had the most significant effect compared to other treatments.

Keywords: Gonad; Hormone; Induction; Laserpuncture; Lobster

Introduction

Currently, spiny lobster is one of the fishery commodities with great potential in Indonesia (Adiyana et al., 2020; Mustafa, 2013). In 2022 based on Yolanda et al. (2022) the demand for sea lobster in Indonesia reached 200,000 tons. However, this does not match the supply of export-ready stocks of sea lobster, such as spiny lobster. It happens because the demand for exports is too high and the availability of lobster stocks is insufficient as written to Erlania et al. (2017). One of the causes is overfishing which can threaten the sustainability of the lobster population in nature (Lesmana et al., 2021). One way to overcome this is to carry out lobster cultivation followed by production engineering treatments such as by making production engineering attempts on spiny lobster broodstock candidates. This can be a solution to the problem of lobster stock availability because efforts to carry out production engineering in this commodity are still rarely carried out (Lante et al., 2018).

Production engineering is one way to increase growth and development in animals (Koshio et al., 1992). One of the latest ways to do production engineering in animals is with laserpuncture. (Kusuma et al. (2017) mentioned that laserpuncture is a production engineering by adapting acupuncture technology that utilizes low waves of laser light so that

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biological tissues are stimulated. This stimulated biological tissue will be able to increase cellular activity related to the growth and development of living things. Hariani (2020) explained that laserpuncture can cause various biological stimulations that can be related to animal reproduction. The use of laserpuncture has also been shown to be safe in some aquatic life compared to other production engineering methods (Mukti et al., 2020).

Laser light induction can affect the reproductive development of aquatic animals such as crustaceans. This is because the crustacean eye stalk or X organ as an induction organ has many nerve receptors that will stimulate the nervous system to forward information to the Y organ whose function is similar to the hypophysis (Shyamal et al., 2018). Then, the Y organ releases gonad development hormones such as CHH (Crustacean Hyperglycemic Hormone), GSH (Gonado Stimulating Hormone), and so on (Budiantoro et al., 2014). The use of laserpuncture has been applied to crustaceans other than lobsters, namely mud crabs. Kusuma et al. (2007) mentioned that the utilization of laserpuncture technology can increase the gonad maturity of mud crabs. Kusuma et al. (2017) mentioned that laserpunktur induction at the reproductive point produces GABA or neurotransmitter. This GABA will stimulate nerves in the hypothalamus which will then produce GnRH which has a role in living things to produce developmental hormones such as Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH). Meanwhile, based on Sainath et al. (2011), states that CHH A has a function to be responsible for the vitelogenesis process in the gonads, while CHH B has a function as a stimulator of oocyte development before the spawning period. This indicates that CHH A in crustaceans has the same function as FSH in vertebrates and CHH B in crustaceans has the same function as LH in vertebrates. Therefore, this study aims to examine the effect of laserpuncture on CHH hormones and gonad histology images as indicators of reproductive development of female spiny lobster.

Method

Experimental Design

This study uses Randomized Experimental Design with 4 treatments with 5 replicates of each treatment. The treatment of this study consists of treatment A, namely induction treatment on female spiny lobster for 2 seconds, treatment B, namely induction treatment on female spiny lobster for 4 seconds, treatment C, namely induction treatment on female spiny lobster for 6 seconds, and control treatment, namely treatment without laserpuncture induction on female spiny lobster. Laserpuncture induction is carried out on the left eye stalk and on the abdomen parallel to the fifth walking leg.

Materials

The object of this research is spiny spiny lobster with weight size 110 ± 5 gr. The female spiny lobster used was acclimatized for 1 week in a 5 x 2 x 1.5 m^3 concrete pond. The device used for the induction treatment of female spiny lobster was a vetenary laserpuncture set at 5 mw, 0.2 cm^2 light output area, and 632.8 nm wavelenght.

Procedures

This research begins with preparing the pond and sea water of female spiny lobster in the form of cleaning treatment on the inner side of the concrete pond. Then, efforts were made to dry the concrete pond to evaporate the remaining residue of the cleaning process. After the drying process, the concrete pond was filled with seawater until the water level reached ± 50 cm from the bottom of the concrete pond. Finally, the PVC shelter is inserted into the concrete tub that has been filled with sterile seawater as a substrate for lobster protection. The step after the preparation of containers and maintenance media is the preparation of spiny lobster. The lobsters used in this research came from the Banyuwangi waters. Before the research, the object in the form of lobsters was given acclimatization treatment for one week.

The induction treatment was carried out once a week within five weeks of the research. Feeding in this study used green mussels (*Perna viridis*) in the amount of 10% of the biomass of female spiny lobster reared (Fadjar et al., 2023). To maintain the water quality of the concrete pond during the research, efforts were made to clean the ponds in the form of flushing which was carried out every two days. CHH hormone data were collected three times, namely before the first laserpuncture induction treatment, in the third week after laserpuncture induction, and at the end of the female spiny lobster rearing period. Sampling of CHH hormone in female spiny lobster is done by taking hemocyte samples on the body of female spiny lobster stored in a heparin tube. CHH hormone is tested using the ELISA method which is read through an ELISA reader with an adsorbance wavelength of 450nm. This based on Huang et al. (2009) which measures CHH levels in another crustacean. Gonad sampling was carried out using a sectio set at the end of the study based on Endryeni et al. (2023). The location of the gonad collection is adjusted to the location of the lobster gonad right between the lobster hepatopancreas. The gonad samples taken are then made into preparations for histological observations made on a microscope with a magnification of 100x. In addition, histological observations were accompanied by scoring of the diameter of female spiny lobster oocytes.

Parameters and Data Analysis

The parameters used in this study were CHH A hormone value, CHH B hormone value, gonad histology (observation and scoring), and survival rate. Scoring is done by measuring the longest diameter of the oocyte vertically and horizontally (Azrifitria et al., 2021). Then the survival rate can be obtained through the following formula based on Amiri et al. (2022):

$$SR\ (\%) = \frac{Nt}{N0} \times 100 \qquad (1)$$

Notes:
SR : Surival Rate
Nt : Final Lobsters Count
No : Initial Lobsters Count

Data analysis in this research used SPSS 26.0. ANOVA test (0.05) was used to determine the effect of laserpuncture on the parameters used and the results of gonadal histology observations according to the treatment were analyzed descriptively.

Result and Discussion

CHH A Hormone

CHH A hormone in female spiny lobster has an increasing growth graph in the third week compared to the first week and a decreasing growth graph in the fifth week. Figure 1 shows that the control treatment has good performance compared to other treatments.

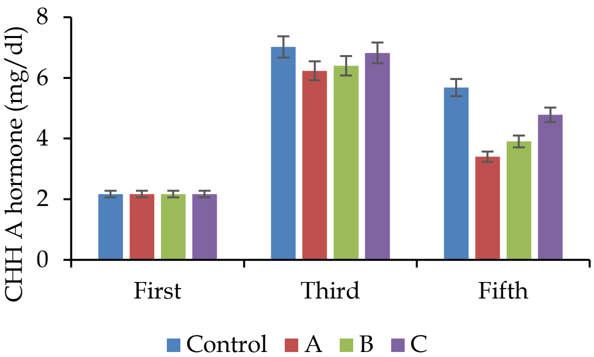


Figure 1. CHH A hormone level

CHH B Hormone

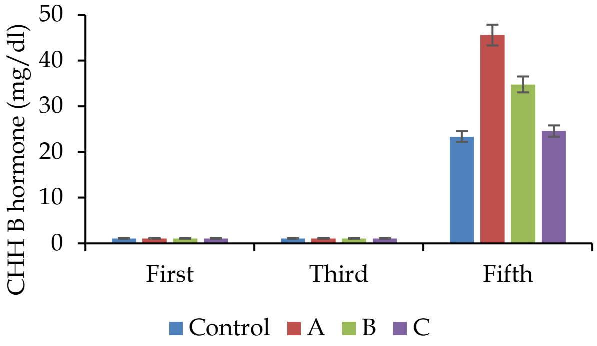


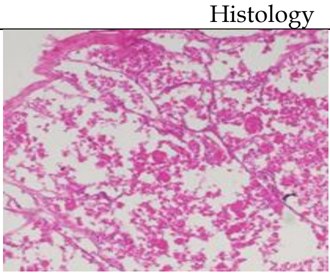
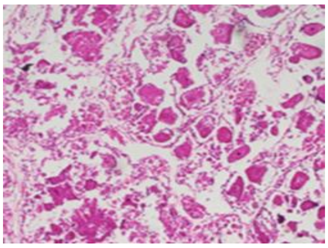
Figure 2. CHH B Hormone Level

CHH B hormone in female spiny lobster has an increasing growth graph in the third week compared to the first week and a decreasing growth graph in the fifth week. Figure 2 shows that treatment A has good performance compared to other treatments.

Gonad Histology

Table 1 shows the histology of the gonads of the control treatment which is dominated by germ cells and a small amount of previtelogenic oocytes, the histology of treatment A gonads is dominated by primary oocytes and secondary oocytes, the histology of treatment B gonads is dominated by previtelogenic oocytes and vitelogenic oocytes, and treatment C is dominated by germ cells and previtelogenic oocytes. While Table 2 shows that the largest oocyte diameter is in treatment A with an average of 122.15 µm.

Table 1. Gonad Histology of Female Spiny Lobster

Treatment	Histology	Characterictics
Control		- germ cell - a small amount of previtelogenic oocytes
C		- germ cell -previtellogenic oocytes

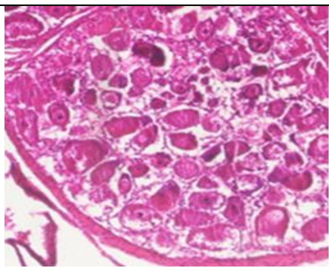
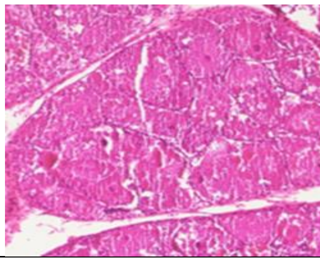
B		-previtellogenic oocytes - vitelogenic oocytes
A		- primary oocyter - a small amount of secondary oocytes

Table 2. Female Spiny Lobster Gonad Scoring

Oocyte diameter (μm)	A	B	C	Control
Average ± SD	122.15[a]± 16.94	96.94[b]± 14.13	88.24[b]± 16.01	69.35[c]± 16.23

Survival Rate

In Table 3, the results of the survival rate are quite high. The survival rate of the control treatment was 90%, treatment A was 90%, treatment B was 85%, and treatment C was 85%.

Table 3. Female Spiny Lobster Survival Rate

Treatment	Control	A	B	C
Survival rate (%)	90[ns]	90[ns]	85[ns]	85[ns]

Discussion

Laserpuncture induction of female spiny lobster (*P. homarus*) with different time durations on gonadal development parameters through the value of CHH A and CHH B levels has a significant effect which can be seen in Table 1 and Table 2. This indicates that laserpuncture applied to spiny lobster causes significant changes in gonad development in female spiny lobster. This is also in accordance with the basic principle that CHH A which is a hormone that is aligned with the hormone FSH will decrease as the gonads develop in living things and CHH B is a hormone that is aligned with the hormone LH or Gonadotropin Hormone (GTH) II, as a hormone to help the process of maturation and release of eggs, will increase as the level of gonadal maturity increases in living things (Rotllant et al., 2018). Supported by the statement of Kusuma et al. (2013) that laserpuncture induction in living things will stimulate the synapse to channel information in the central system of living things to produce the neurotransmitter GABA. GABA has several functions in crustaceans, one of which is to produce various hormones related to spiny lobster development as in Berliyantia et al. (2020). This GABA will synthesize GTH I to be channeled to the gonads so that the vitellogenin development process occurs before the gonads mature according to Lee et al. (2014). Induction of laserpuncture in living things will also stimulate the synapse to channel information to the living things' central system to produce the neurotransmitter GABA. This is in accordance with Li et al. (2016) which states that biological stimulation can produce neurotransmitter GABA in living things such as crustaceans. This GABA will synthesize GTH II to be transmitted to the gonads for the final egg maturation process (Biscocho et al., 2018). In addition, GTH II can also stimulate spawning in spiny lobster (Kusuma et al., 2013). Meanwhile, de Kleijn et al. (1998) stated that the increasing value of gonado somatic index in *Panulirus americanus*, the value of CHH A hormone will decrease. This is because CHH A functions in crustaceans as a gonadotropin hormone before the spawning period so that if living things are almost entering the gonadal maturity phase, the synthesis of this hormone will be inhibited slowly. So that the value of this hormone will decrease along with the maturity of the female spiny lobster gonads (Chung et al., 2010).

This proves that the control treatment has high levels of CHH A hormone compared to other treatments because lobsters that are not induced are still in the process of vitellogenin development, which is the process of oogenesis which is still in the stage before the formation of oocytes. Meanwhile, based on Chen et al. (2020) stated that the increasing value of crustacean ECG, the value of CHH B hormone also increases. This is because the function of CHH B in crustaceans is a hormone that is active to stimulate oocyte formation. So that the value of the hormone also rises along with the increase in the level of gonadal maturity and gonado somatic index of female spiny lobster (Loredo-Ranjel et al., 2017). This also explains why the movement of the CHH A hormone value decreases every week and the CHH B hormone value increases every week. It happens because the function of CHH A is more active first for the process of gonadal vitelogenesis followed by the active CHH B which is utilized for the process of oocyte maturation in female spiny lobster (Pamuru, 2019).

This research also proves that laserpuncture can effectively accelerate the maturity of female gonads in aquatic life, especially female spiny lobster which can be seen in Table 1 and Table 2. This increase can occur due to laser induction at the reproductive point in the form of electromagnetic waves, which will then penetrate the epidermis which is rich in peripheral nerve endings (Hartenstein, 2006). The energy from the electromagnetic waves will be converted into electrical signals in the nerve cell membrane. The existence of electrical signals will result in depolarization of the nerve cell membrane. The effect of depolarization of the nerve cell membrane will produce impulses that will be forwarded to the brain, then will stimulate the hypothalamus to secrete Gonadotropin Releasing Hormone (GnRH), as explained in Duitoz et al. (2021).

GnRH will release CHH A and CHH B hormones that can accelerate the development of female spiny lobster gonads (Kusuma et al., 2017). This indicates that laserpuncture exposure at the reproductive point can improve the performance of hormones which are the reproductive control system in accelerating the growth, development and maturation of lobster gonads, one of which is through observations of gonad histology and measuring the diameter of female spiny lobster oocytes (Kusuma et al., 2017).

This is evidenced in histological observations in each laserpuncture induction treatment given. The control treatment gonads are in the early developing phase. Ikhwanuddin et al. (2015) explained that the presence of germ cells and previtelogenic oocytes that are dominant in the gonads indicates that the gonads have completed the immature phase and are entering the early developing phase. Treatment A gonads are in the spawning capable phase. Yusnaini et al. (2019) explained that lobster gonads have entered the final stage of gonad development when there are primary oocytes and secondary oocytes. Secondary oocytes will later be released by female spiny lobster into eggs stored in the swimming legs of the lobster. After that, they will be released and spawned by male spiny lobster sperm cells (Kagwade, 1988). Treatment B gonads are in the late developing phase. Atherley et al. (2021), stated that lobster gonads have entered the late developing stage when previtelogenic oocytes and vitelogenic oocytes are found in the lobster gonads. This is also in accordance with Aretto et al. (2008), which explains that lobster gonads have entered the late development stage when the lobster gonads have vitelogenic oocytes in them. While treatment C gonads are in the early developing phase. Mota (1965) explained that the early developing phase of gonads in the genus Panulirus is characterized by the appearance of previtelogenic oocytes, but there are still a small portion of germ cells that exist in the gonads of female spiny lobster. In histology based on Minagawa et al. (1995), the early developing phase on lobster is characterized by the appearance of previtellogenic oocytes in the gonads.

However, laserpuncture induction on female spiny lobster has no significant effect on the survival of female spiny lobster as shown in Table 3. Nevertheless, the application of laserpuncture induction on female spiny lobster is still safe to be applied to this commodity. This is supported by the opinion of Amiri et al. (2022) which states that a high survival rate indicates that the level of cannibalism of spiny lobster is low. Therefore, if the survival rate in rearing activities is relatively high, it can be said that the activity is feasible (Prariska et al., 2020). Many factors can affect the SR value. According to Amrillah et al. (2022), some of these factors are adaptation, feed, and water quality. Besides the laserpuncture treatment, the maintenance of female spiny lobster needs to be maintained in terms of adaptation, feeding, and water quality. Laserpuncture treatment can certainly affect the adaptation pattern of female spiny lobster. Therefore, to keep the adaptation pattern stable, it is necessary to maintain feeding according to nutritional needs and feeding time and also maintain water quality (Ratunil et al., 2017). By maintaining these aspects, the lobsters can be ensured not to experience excessive stress which can increase cannibalism of female spiny lobsters and decrease the mortality rate as described by Cruz et al. (2020).

Conclusion

The conclusion obtained in this research is that laserpuncture induction can be utilized to accelerate the development of female spiny lobster gonads. This can be evidenced from the CHH hormone parameters and gonad histology in female spiny lobster which are significant when induced by laserpuncture. Based on this study, the recommended induction treatment is laserpuncture induction on the eye stalk and ventral part of female spiny lobster for 2 seconds using vetenary laserpuncture.

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Conceptualization, Wibowo, J. C., Fadjar, M., and Maimunah, Y.; methodology, Wibowo, J. C.; validation, Fadjar, M., and Maimunah, Y.; formal analysis, Wibowo, J. C.; investigation, Wibowo, J. C.; resources, Wibowo, J. C., Fadjar, M., and Maimunah, Y.; data curation, Wibowo, J. C.; writing—original draft preparation, Wibowo, J. C.; writing—review and editing, Wibowo, J. C.; visualization, Wibowo, J. C.; supervision, Fadjar, M., and Maimunah, Y.

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Conflicts of Interest
The authors declare no conflict of interest.

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