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The Effect of Eye-stalk and Anti-dopamine on Number of Eggs and Nauplii Production in Vannamei Shrimp (*Litopenaeus vanammei*)

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Abstract. One of the shrimp commodities in high demand on the global market is the vannamei shrimp (*Litopenaeus vannamei*), and Indonesia is one of the leading countries in shrimp production. To improve the growth and quality of vannamei shrimp production, farming techniques continue to be developed. The practice of eye-stalk cutting on shrimp, also known as eyestalk-cutting, has become a technique actively employed in shrimp farming. However, it is considered a violation of animal welfare regulations. Anti-dopamine is another alternative that can inhibit the gonad maturation inhibiting hormone and improve shrimp reproductive performance. This study aims to determine the effect of eye stalk and anti-dopamine injection on the comparison of egg and nauplii production as well as the hatching rate carried out at Balai Produksi Induk Udang Unggul dan Kekerangan (BPIU2K) in Karangasem, Bali. The research method employed was experimental, with data analysis conducted using an independent t-test. The results showed that eye-stalk produces ±269,000 eggs with a hatching rate (HR) of 26.13%, while anti-dopamine can produce up to ±91,600 eggs with an HR of 83.31%. Total nauplii production from eye-stalk treatment reached ±75,000, and anti-dopamine nauplii production reached ±77,100. T-test results with a 95% significance level indicate a difference in the number of eggs but no significant difference in total nauplii production.

Keywords: anti-dopamine; eye-stalk; hatch rate; vanammei shrimp

I. INTRODUCTION

Indonesia is one of the top five leading countries in capture fisheries and crustacean aquaculture, with a total production of 1,290.2 thousand tons in 2020 [1]. One of the shrimp commodities in high demand in the global market is the Vannamei shrimp (*Litopenaeus vannamei*) [2]. The reproductive development of Vannamei shrimp is influenced by the Gonad Inhibiting Hormone (GIH) produced in the eyestalk of the shrimp [3]. The eyestalk technique is commonly adopted as a practice to increase shrimp seed production in response to market demand urgency [4]. It involves the procedure of removing the Gonad Inhibiting Hormone by cutting the eyestalk of the shrimp, which accelerates the gonad maturation process [3].

The eye-stalk procedure has begun to receive attention and opposition due to concerns about animal welfare [5]. The majority of developed countries, being the world's largest importers, have made animal welfare a fundamental requirement in the trade of aquatic organisms [6]. The eye-stalk technique is known to cause trauma and stress in shrimp after the procedure is performed [7], and it also increases the probability of reducing the quality and quantity of seedlings and parental reproduction [8].

Anti-dopamine is considered an alternative method, with several observations showing positive results in the reproductive development of shrimp. Hormone induction using PMSG (Pregnant Mare Serum Gonadotropin) injection and anti-dopamine can enhance sperm quality by producing a high percentage of normal sperm with low abnormality in male shrimp [8].

The purpose of this research is to investigate the effects of eye-stalk practice and the administration of anti-dopamine on the comparison of egg quantity with nauplii production and the hatching percentage of Vannamei shrimp larvae eggs. This aims to conduct further studies on improving the production of high-quality shrimp seeds while minimizing practices that contradict animal welfare regulations.

II. RESEARCH METHODS

A. Time and Location

This research was conducted at Balai Produksi Induk Udang Unggul Dan Kekerangan (BPIU2K), Shrimp Unit, Desa Bugbug, Kabupaten Karangasem, Bali. The study was conducted over two months, from November 2022 to January 2023.

B. Equipment and Materials

The research instruments used were Maintenance tanks, which also served as broodstock spawning tanks; hatchery tanks; basins; a 10 µm sieve; a pH meter; a refractometer; a thermometer; a Dissolved Oxygen (DO) meter; scissors; and a 1 ml Syringe. The research materials used included female and male vannamei shrimp broodstock, each consisting of 50 individuals, Domperidone (an anti-dopamine agent), and distilled water (Aquadest).

C. Research Methods

The research method employed was a descriptive, quantitative approach with an experimental data collection method. The research stages included the preparation of containers and maintenance media, acquisition and selection of broodstock, water quality management, practices of eye-stalk and anti-dopamine injection, gonad maturation and spawning process, as well as egg and nauplius counting.

D. Research Parameters

The data obtained in this research include survival rate, weight growth, gonad maturity level, and spawning success rate of broodstock, as well as the total number of eggs and nauplii. A comparative analysis was conducted between the number of eggs produced and the number of nauplii using a T-test with SPSS v.29 software.

a. Broodstock Survival Rate

Broodstock survival rate represents the total of all broodstock from beginning to the end of the research period. The survival rate of Vannamei shrimp broodstock can be calculated using the following formula [9]:

$$SR = \frac{N_t}{N_0}\ x\ 100\ \%$$

Noted: SR is the Survival Rate (final number of individuals/initial number of individuals); *Nt* is the total number of shrimps at end period (individuals); and N_0 is the total number of shrimps at the start of research (individuals).

b. Broodstock Weight Growth

Absolute weight growth of the test shrimp can be calculated using the formula according to reference [10]:

$$W_m = W_t - W_0$$

Noted: Wm is the absolute weight growth (average weight of individuals at the end of research /average weight of individuals at the start of research); Wt is the average shrimp weight at the end of research (grams); and W0 is the average shrimp weight at the beginning of research (grams).

c. Gonad Maturity Level Growth

The growth of gonad maturity levels is monitored daily, from the second week to the end of the research period, through direct observation. This involves assessing the color intensity of gonads on the back of the female shrimp to determine the maturity level. Calculations are performed on all shrimp, with data recorded from gonad maturity levels 1 through 4. The gonad maturity of the shrimp is characterized by a yellowish-red color on the back of the broodstock.

d. Spawning Success Rate

Successful spawning broodstock is characterized by the attachment of the male's spermatophore to the female's lyceum, after which the successful spawning broodstock is transferred to the egg release & hatching tank. The percentage of the spawning success rate can be calculated using the following formula [9}:

$$Tp = \frac{Spawning\ broodstock}{Total\ combined\ female\ broodstock}\ x\ 100\ \%$$

Noted: *Tp* is percentage of the spawning success rate (number of spawning broodstock/total broodstock per treatment); *Spawning broodstock* is number of broodstock that succeeded in spawning (individuals); and *Total combined female broodstock* is the total number of broodstock in that particular treatment (individuals).

e. Egg Quantity

Total egg count from each broodstock can be calculated using a sampling method with the following formula [9]:

$$Jt = \frac{Bp}{Ps\ x\ Gc}\ x\ Yt$$

Noted: Jt is the number of eggs produced by each broodstock (tank volume/sample frequency x volume of glass) x number of eggs); Bp is the volume of the spawning tank (ml); Ps is the frequency of egg sample collection; Gc is the volume of water in the measuring cup during the sample collection of spawning eggs (ml); and Yt is the total number of eggs from all samples (units).

f. Hatching Rate

Egg hatching percentage is one of the key indicators of shrimp hatchery performance [11], influenced by various factors, including egg quality, temperature, dissolved oxygen, and pH [12]. The hatching rate can be calculated using the formula [13]:

$$HR = \frac{Number\ of\ eggs\ hatched}{Total\ eggs\ in\ tank}\ x\ 100\%$$

Noted: HR is hatching percentage (hatched eggs/initial egg count); The number of eggs hatched is the number of eggs that have hatched into nauplii (individuals); and the Total number of eggs in the tank is the total number of eggs released by the broodstock (units).

g. Independent T-Test

T-test is a parametric statistical test method [14] indicating the extent to which one independent variable affects the dependent variable [15]. The testing mechanism for accepting or rejecting the hypothesis with a 95% confidence interval ($\alpha = 0.05$) is as follows:

1. T-test on the egg count results
   - H_0 = There is no significant difference in egg count from the broodstock subjected to either the eye-stalk treatment or the anti-dopamine injection treatment.
   - H_1 = There is a significant difference in egg count from the broodstock subjected to either the eye-stalk treatment or the anti-dopamine injection treatment.
2. T-test on the nauplii count results
   - H_0 = There is no significant difference in nauplii count from the broodstock subjected to either the eye-stalk treatment or the anti-dopamine injection treatment.
   - H_1 = There is a significant difference in nauplii count from the broodstock subjected to either the eye-stalk treatment or the anti-dopamine injection treatment.

The rules for decision-making are as follows:
If probability > 0.05, then H_0 is accepted.
If probability < 0.05, then H_0 is rejected.

III. RESULT AND DISCUSSION

Result

a. Broodstock Survival Rate

The bservation results (Figure 1) show a decrease from 96% in the 3rd week to 88% in the 4th week of maintenance, with a total of three deaths among the eye-stalked broodstock throughout the study. Meanwhile, the broodstock given anti-dopamine maintained the same number until the end of the study, indicating a 100% survival rate.

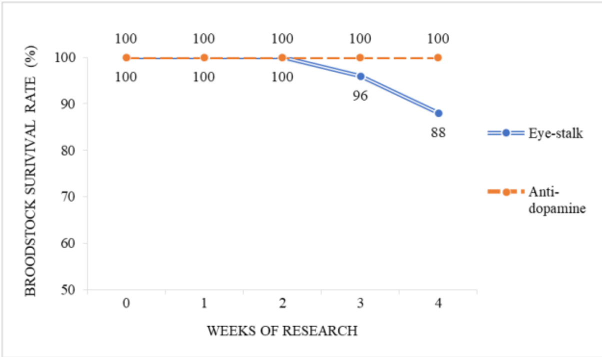

Figure. 1. Survival Rate of Vannamei Broodstock of one-month research.

b. Broodstock Weight Growth

Referring to Figure 2, the highest weight growth of female broodstock was found in shrimp given the anti-dopamine treatment, with an increase reaching up to 13.16 grams over 4 weeks of maintenance and an average increase of 3.29 grams. This was despite a decrease of an average of 0.29 grams during the 2nd week of maintenance. The weight of shrimp given eye-stalk treatment decreased until the 2nd week, then increased by 2.86 grams until the end of the research.

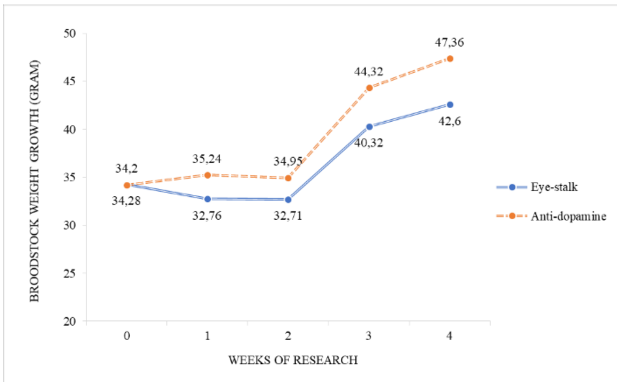

Figure. 2. Broodstock Weight Growth over one-month research.

c. Gonad Maturity Level Growth

No hrimp had matured gonads during the 1st to 2nd week of research across all treatments. The development of mature gonads in shrimp began to appear as maintenance progressed into the 4th to 5th week, with the number of mature gonad shrimp reaching 17 for the anti-dopamine treatment (Figure 3.a), while all eye-stalk-treated shrimp (Figure 3.b) achieved gonad maturity (25 individuals).

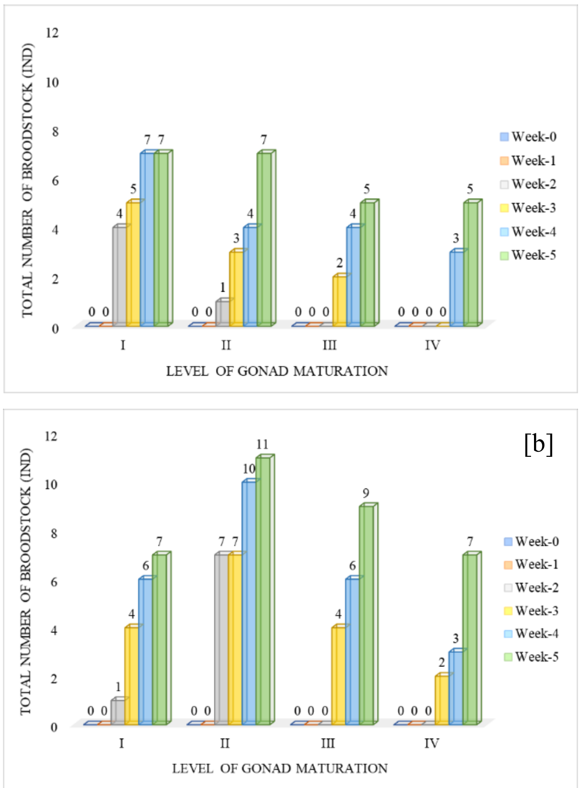

Figure. 3. Growth Chart of Broodstock. (a) Anti-dopamine Broodstock, (b) Eye-stalk Broodstock.

d. Spawning and Fertilization Success Rate

The highest percentage of spawning success was observed in shrimp treated with anti-dopamine, reaching a success rate of 65%, and the eye-stalk treatment had a success rate of 32% (Table 1). The percentage of successful fertilization of eggs is shown in Table 2, with eye-stalked shrimp achieving the highest percentage of 63%, while the anti-dopamine-treated shrimp attained a rate of 45%.

TABLE 1
SPAWNING RATE

Treatment	Spawning Rate		Success Rate
	Gonad Matured (ind)	Spawned (ind)	
Eye-stalk	25	8	32%
Anti-dopamine	17	11	65%

TABLE 1
FERTILIZATION RATE

Treatment	Fertilization Rate			Success Rate
	Gonad Matured (ind)	Spawned (ind)	Laid Eggs (ind)	
Eye-stalk	25	8	5	63%
Anti-dopamine	17	11	5	45%

e. Number of Eggs, Nauplii, and Hatching Rate

In anti-dopamine treatment, the average number of eggs produced was around 91,600 eggs, while the eye-stalked shrimp still obtained the highest average egg total, reaching 126,000 eggs. However, in both treatments, the average number of nauplii found was not significantly different, with an average of 75,000 nauplii for the eye-stalked shrimp and a slightly higher count for the anti-dopamine shrimp, at an average of 77,100 nauplii. The average hatching rate (HR) differed significantly between the two treatments. The shrimp given anti-dopamine treatment had a higher average HR of 83.31%, while the shrimp given eye-stalk treatment had a lower HR with a percentage of 26.13% (Table 3).

TABLE 3
AVERAGE NUMBER OF EGGS AND NAUPLI PRODUCTION

	Average eggs	Average Nauplii	Average *HR* (%)
Eye-stalk	269.600 ± 52.218	75.000 ± 47.754	26,13 %
Anti-dopamine	91.600 ± 30.311	77.100 ± 28.400	83,31 %

The T-test calculation results, obtained from the comparison of significance at a 95% confidence level, yielded a result of 0.001 < 0.05, indicating acceptance of the alternative hypothesis (H1) that there is a significant difference in the number of eggs produced by the two treatments. For the variable of the number of nauplii, the result obtained was 0.0935 > 0.05, thus accepting H0, which means there is no difference in the number of nauplii from the two treatments.

Discussion

a. Comparison of the Effects of Eye-stalk and Anti-dopamine on Egg Production.

The verage number of eggs produced was highest in the eye-stalk treatment, with an average of approximately 269,600 eggs, while anti-dopamine resulted in an average of 91,600 eggs. The T-test on the number of eggs from both treatments with a 95% confidence interval yielded a result of 0.001 < 0.05, indicating acceptance of the alternative hypothesis (H1) that there is a significant difference. The significant difference in egg production is closely related to gonad maturity development, where the anti-dopamine-treated shrimp reached 17 mature individuals compared to the total of 25 individuals for the eye-stalk treatment by the end of the study. This suggests that diversification of fresh feed in high protein played a crucial role in gonad maturation [16]. Additionally, the appropriate application of the gonad maturation development technique is indicated as the basis support for all eye-stalked broodstock in this study to mature their gonads relatively faster than other treatments.

It was noted until the end of the study that the Survival Rate (SR) of eye-stalk shrimp (88%) was lower compared to the anti-dopamine shrimp (100%). The post-eye-stalk stress is speculated to be the trigger for weight loss and mortality of broodstock. The eye stalk played a role in lowering glucose levels, resulting in disrupted glucose metabolism and the production of energy under stress conditions [17], which caused the energy metabolism from feed absorption to be allocated to physical recovery [18]. However, the eye-stalk treatment showed a superior fertilization success rate (63%) compared to the anti-dopamine (45%). The weight increase observed in the last two weeks of the study is supported by the fact that eyestalk removal is an effective technique for developing shrimp gonad maturation, as it involves the direct removal of the gonad-inhibiting hormone found in the eyestalk, which may underlie this phenomenon. Research by reference [5] also yielded similar results regarding the success rate of spawning and egg production, with eye-stalk-treated shrimp exhibiting higher rates but lower fecundity rates over the observation period.

b. Comparison of the Effects of Eye-stalk and Anti-dopamine on Nauplii Production.

anti-dopamine treatment showed a superior average nauplii count of 77,100 nauplii, while the eye-stalk treatment had an average of 75,000 nauplii. The T-test result of 0.0935 > 0.05 accepted H0, indicating no difference in the number of nauplii between the two treatments. This is likely due to the lower stress level in anti-dopamine shrimp, as evidenced by several indicators, including the 100% survival rate of the anti-dopamine shrimp until the end of the study and the weight loss experienced only during the third week of research. This is in line with reference [9], where the highest average weight increase occurred in shrimp that did not receive eye-stalk treatment, and conversely, eye-stalked shrimp experienced weight loss during the 2nd to 3rd week of research.

Several factors and indicators are believed to support the higher spawning rate of anti-dopamine shrimp, reaching 65% compared to 32% for the eye-stalked shrimp. Another factor that promotes the high spawning success rate is the frequent mating frequency between male and female broodstock, as well as the handling technique employed during the transfer of spawning broodstock to the hatching tank. However, the anti-dopamine treatment tends to have a lower fertilization rate (45%) compared to the eye-stalk treatment (63%), as stated in reference [19], which notes that one of the factors is the ability of male shrimp sperm, closely related to fertilization. The phenomenon may be related to the sperm attachment position, which tends to detach easily from the telecom during the transfer process of successfully spawning anti-dopamine broodstock to the spawning tank due to lower stress levels, resulting in more active behavior and less effective fertilization.

In this study, the comparison of the hatching rate (HR) between the two treatments reveals a considerable difference, with 26.13% for the eye stalk treatment and 83.31% for the anti-dopamine treatment. A study by reference [20] supports this, stating that over time, the egg and nauplii production in constant eye-stalk treatment changes when compared to shrimp that did not receive any treatment, resulting in higher egg production and a higher hatching rate due to gonad maturation and development over a more extended period. Meanwhile, the eye-stalk treatment can stimulate gonad maturation to an excessive level in a short period, leading to a decrease in egg quality, which is one of the factors contributing to the reduction in the hatching rate.

III. CONCLUSION

Eye-stalk treatment influences the development of gonad maturation and excels in egg production quantity, yet it results in a low hatching rate. On the other hand, the anti-dopamine injection treatment effectively increases the chances of egg development having a high hatching rate. The results of the test indicate that both treatments have a significant difference in the number of eggs, but there is no significant difference in the outcome of nauplii production.

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