International Journal on Advanced Science Engineering Information Technology

Vol.14 (2024) No. 4
ISSN: 2088-5334

Efficacy of *Cyperus* spp. Extract Components in Improving Reproductive Performance of Captive Tiger Shrimp (*Penaeus monodon*)

Emma Suryati [a,1], Rosmiati Rosmiati [a,2], Andi Parenrengi [a], Rachman Syah [a], Sulaeman Sulaeman [a], Andi Tenriulo [a], Samuel Lante [a], Agus Nawang [a], Makmur Makmur [a], Elmi Nurhaidah Zainuddin [b,3]

[a] *Research Center for Fisheries, National Research and Innovation Agency, Indonesia*
[b] *Faculty of Marine Science and Fisheries, University of Hasanuddin, Makassar, South Sulawesi, Indonesia*
Corresponding author: [1] emmasuryati@yahool.com; [2] rosm005@brin.go.id; [3] elmi18id@gmail.com

***Abstract*—The reproductive performance of captive tiger shrimp (*Penaeus monodon*) is a crucial aspect of sustainable aquaculture. This study explores the potential of Cyperus spp. extract components (CE) to enhance the reproductive performance of captive tiger shrimp Penaeus monodon. Our methods involved feeding captive tiger shrimp Penaeus monodon with feed enriched with 0, 300, 400, and 500 µg CE/g Body Weight (BW) over a 60-day rearing period. Eyestalk ablation was used as a positive control. Post-feeding experiments, we observed the shrimp for their reproductive performance. The results were promising: the male broodstock exhibited the highest molting percentage when fed with feed enriched with 400 µg CE/g BW, while females showed the highest molting percentage at 500 µg CE/g BW. The highest percentage of males carrying spermatophores and mating females was observed at 500 µg CE/g BW. Broodstock-fed feed enriched with 500 µg CE/g BW produced 337,339 eggs with an average egg diameter of 289.5 µm, yielding 16,175 nauplii. The highest expression of vitellogenin and spermatogenesis was found in the 400-500 µg/g BW range. These findings suggest that the optimal dose of CE for promoting gonadal maturation in captive shrimp broodstock is 400-500 µg/g BW. The potential of this study to increase larval production in aquaculture practices is significant. By utilizing environmentally friendly plant-derived hormones like CE, we can develop more sustainable aquaculture methods, thereby reducing the environmental impact associated with traditional hormone induction strategies.**

***Keywords*—*Cyperus* spp.; Methyl farnesoate; oral administration; reproductive performance, tiger shrimp.**

Manuscript received 22 Dec. 2023; revised 10 May 2024; accepted 20 Jun. 2024. Date of publication 31 Aug. 2024.

I. INTRODUCTION

Tiger shrimp Penaeus monodon is an aquaculture industry that has become the second largest leading commodity in the world [1]. In 2023, the production of tiger shrimp reached approximately 550,000 metric tons, and it is anticipated to increase to 600,000 metric tons by the year 2024 [2]. To achieve the target of sustainable tiger shrimp farming, sufficient high-quality shrimp broodstock is required for the provision of seedlings [3]. Until now, the availability of tiger shrimp broodstock for spawning still depends on natural broodstock. However, there is a high risk of disease transmission, potentially resulting in disease outbreaks [4], [5]. Efforts have been made to replace wild tiger shrimp broodstock with captive-bred broodstock; however, utilizing captive-bred broodstock still poses several challenges, such as poor reproductive performance. Poor reproductive performance is associated with the slow maturity of shrimp gonads, leading to low or even inability to perform natural mating in controlled ponds or tanks [6], [7].

Eye stalk ablation has been shown to enhance the captive tiger shrimp broodstock's reproductive performance [8]–[10]. Although eyestalk ablation can accelerate tiger shrimp gonad development, this method has drawbacks, such as increasing stress levels that can result in spawner death, disrupting physiological function, reducing gamete quality, and contrary to animal welfare [11]–[13]. Hormone induction strategies offer an alternative avenue to ensure the availability of endogenous steroid hormones essential for regulating reproductive activity [14]–[16]. While effective, synthetic steroid hormones come with high-cost challenges and environmental concerns, including potential residues they may leave in aquatic environments [17], [18].

Hormones are derived from environmentally friendly plants, so they are safe to use to enhance the tiger shrimp broodstock's reproduction. *Cyperus* spp., one of the wild plants that often grows in fields, rice fields, and beaches, has significant potential in aquaculture. *Cyperus* spp. contain various bioactive compounds, including alkaloids, terpenes, phenolic acids, and sesquiterpenes, like methyl farnesoate (MF) [18], [19]. MF found in *Cyperus* spp. has similarities with juvenile hormones present in crustaceans [19], and this opens opportunities to stimulate hormonal responses that can enhance tiger shrimp reproduction in hatchery settings.

The influence of the use of *Cyperus* spp. extract components (CE) on the captive tiger shrimp broodstock's reproductive performance is still in the research stage. In the earlier study, oral administration of CE to captive tiger shrimp broodstock improved gonadal development and egg production, especially at concentrations of 100 µg/g body weight (BW) through four weekly injections [20]. However, the practical challenges and risk of death associated with injection feeding have triggered a shift towards mixing feeding with feed. In the application of CE at a concentration of 300 µg/g BW through feed, an increase in reproductive performance was found [21]. However, it has not provided significant results, so appropriate concentration optimization is needed. Thus, this investigation aims to determine the impact of oral administration of CE at various doses on the captive tiger shrimp broodstock's reproductive performance. The percentage of molting, the number of males carrying spermatophores, mated females, the development of Gonadal Maturity Level (GML), the number and diameter of eggs, and the number of nauplii of captive tiger shrimp broodstock were evaluated. The findings from this study have the potential to contribute to the development of more efficient captive tiger shrimp farming methods and increase larval production while reducing the use of synthetic chemicals and reducing the need for eyestalk ablation techniques. This research aims to evaluate the effectiveness of extracts from Cyperus species in enhancing the reproductive capacity of tiger shrimp bred in captivity.

II. Material and Methods

A. *Cyperus spp. Extraction*

Cyperus spp. was collected around agricultural land in Bontojolong Village, Maros Regency, Indonesia. The rhizome of Cyperus spp. was separated and then washed under running water to remove any soil that was still attached. The clean Cyperus spp. rhizome was then cut into cubes and dried using a drying cabinet at 40°C. The dried Cyperus spp. was ground into a fine powder using a blender. The extraction process is shown in Fig. 1. The concentrated extract was recrystallized with chloroform to get the *methyl farnesoate* mixture [21].

B. *Shrimp Rearing*

The broodstock of captive tiger shrimp (*Penaeus monodon*) was purchased from a traditional pond located in the Takalar Regency, South Sulawesi, Indonesia. Previously, the broodstocks were confirmed to be free of specific pathogens based on a polymerase chain reaction (PCR) result. The shrimp broodstocks were transported in plastic bags and gradually acclimatized to laboratory conditions. They were provided with a commercial diet for approximately seven days before the commencement of the experiment. Healthy female broodstocks with a body weight and body length ranging from 104.56-117.25 g and 19.00-22.95 cm, and male broodstocks with a body weight and body length ranging from 69.12-79.50 g and 19.21-20.66 cm, respectively were selected. The broodstocks in an empty stage of Gonadal Maturation Level (GML) were selected and randomly distributed into ten concrete tubs, each having a volume of 20 tons. Each tub has a recirculation water system with a shrimp density of 8 pairs (n=16).

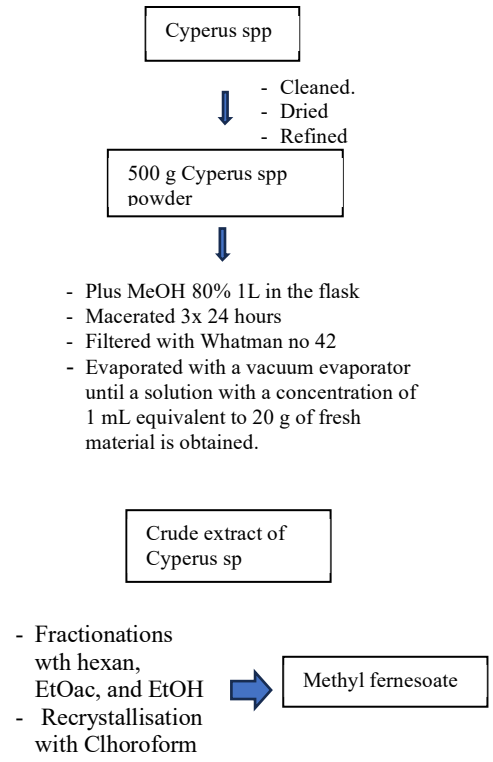

Fig. 1 *Cyperus spp.* extraction process

Each broodstock was given conventional numbering and marking to one of the eyepieces and carapace. The broodstocks were fed squid, clams, and sea worms twice a day, namely morning and afternoon, as much as 15% of the shrimp's body weight. Before administration, the feed was soaked in a 100-ppm iodine solution for 10 minutes to disinfect the feed. In addition, the broodstocks were fed a commercial diet of as much as 2% of body weight daily at night. A weekly water exchange of about 50% was performed using filtered and sterilized seawater to maintain water quality.

C. *Ethics Statement*

The research met the ethical standards for animal welfare, as established by the UMI Health Research Ethics Commission, documented with reference number 205/A.1/KEPK-UMI/I/2022 (January 10, 2022).

D. *Experimental Diet Preparation*

The extract from Cyperus sp. the extract was incorporated into commercial shrimp feed (PT Central Proteina Prima Tbk) in the form of crumble with a crude protein content of 40%

according to the designed treatment dose. The feed enriched with CE was coated with fish oil, with a dosage of 15 mL/kg, to enhance the extract's stability and effectiveness. The coating was sprayed [22]. After the coating process, the experimental feed was dried and stored in a refrigerator at 4°C until ready for use.

E. Cyperus spp. *Extract Administration*

CE was given through oral administration using a Completely Randomized Design (CRD). This study involved five different treatments. The captive tiger shrimp *Penaeus monodon* was fed feed enriched with 0 (A), 300 (B), 400 (C), and 500 µg CE/g BW (D). Another treatment (E) was eyestalk ablation as a positive control. All treatments were conducted in duplicates. The male and female broodstocks were fed commercial feed enriched with CE based on the designed treatment concentration. The broodstocks were fed a commercial feed enriched with CE as much as 2% of the total body weight of shrimp at night in addition to fresh feed in the morning and afternoon for 60 days of the rearing period.

F. Parameter Observation

The molting of tiger shrimp broodstock was monitored daily following the application of CE. Molting was identified by the shedding of the old cuticle and the subsequent regeneration of a new one in the carapace of tiger shrimp [23]. The molt percentage for both females and males was determined by comparing the count of molting shrimp to the total number of shrimps examined. The males carrying spermatophores were observed daily. The presence of mating female broodstock was monitored daily. The number of eggs produced, average egg diameter, and nauplii yield were recorded.

The female tiger shrimp broodstock generally reached gonad maturity within 7-10 days after ablation. Observation of mature broodstock gonads was carried out every evening (5.00-6.00 Indonesia time) by shining a part of the shrimp body. A GML IV was characterized by an even spread of gonads visible from the carapaces of the dorsal aspect of the body to the base of the tail [24]. Shrimp showing the GML IV were transferred into a conical spawning tank with a capacity of 250 L for hatching. The number and diameter of eggs and the number of nauplii were observed. At the end of the study, the vitellogenin and spermatogenesis expression were observed in the broodstocks.

To determine the gene expression of female broodstock vitellogenin and male broodstock gametes was done by isolating RNA using RNA isolation kits to synthesize cDNA to be used as DNA temples [25]. The RNA was extracted from 100 µL hemolymph using an RNA. isolation kit and cDNA synthesis using Ready-To-Go. was extracted from 100 µL hemolymph using an RNA. isolation k

G. Data Analysis

The data on molt percentage, the count of males with spermatophores, and mated females underwent Analysis of Variance (ANOVA) at a confidence level of 0.05 ($P<0.05$). Microsoft Excel and IBM SPSS Statistics 20 were employed as computer software for analysis. Descriptive presentations were given for the development of GML, egg count, diameter, and nauplii count. The discussion included a descriptive examination of the expression of the gene responsible for vitellogenin and spermatogenesis in the hemolymph of female and male tiger shrimp.

III. Results and Discussion

A. Shrimp Molting

Applying CE at the different concentrations on the tiger shrimp broodstock showed the response in males and females, especially in the molting process. Data on shrimp molting percentage during the study are presented in Figure 2.

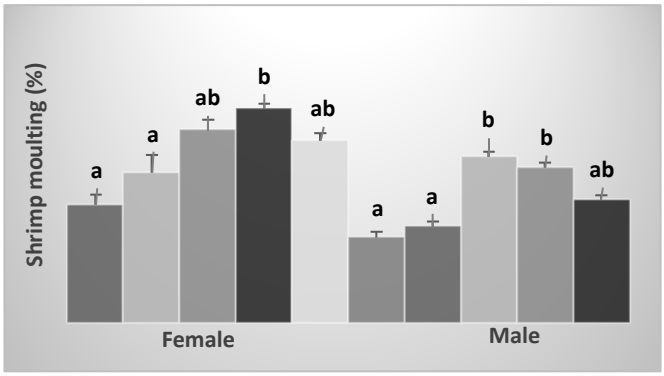

Fig. 2 Molting percentage of tiger shrimp broodstock after application of CE treatment. A=control/without extract (0 µg/g BW); B=300 µg/g BW, C=400 µg/g BW; D=500 µg/g BW, E=eyestalk ablation; and the values with different letters were significantly different ($P<0.05$

You-Prime First Strand Beads kit. DNA amplification using the PCR technique, regarding the method previously developed by Parenrengi et al.[26]. The cDNA findings serve as the DNA templates for PCR amplification in assessing the expression of vitellogenin and gametogenesis genes. The tiger shrimp β-actin gene expression as a control gene expression. The tiger shrimp β-actin gene expression as a control gene expression. The standard protocol for gene amplification involved utilizing a PCR machine. The PCR outcomes were subjected to electrophoresis on a 1.0%. agarose gel and recorded using the Gel Documentation System. The detection of a specific DNA fragment in the gel signifies the expression of these genes, with approximately. 50 base pairs for females and around 200 base pairs for males.

During 60 days of maintenance, the female broodstock exhibited the highest molting percentage when fed with feed enriched with CE at the concentration of 500 µg/g BW. On average, the molting percentage in this concentration treatment significantly differed ($P<0.05$) from the female broodstock fed with feed enriched with CE at the concentration of 300 and 0 µg/g BW (without CE enrichment) but did not show a significant difference ($P>0.05$) to the treatment 400 µg/g BW and eyestalk ablation. It was different from the response shown by the male broodstock. The highest molting percentage was obtained in the male broodstock fed CE-enriched feed at the concentration of 400 µg/g BW and did not exhibit a significant difference ($P>0.05$) between 500 µg/g BW and eyestalk ablation. However, the males treated with CE-enriched feed at 400 µg/g BW significantly differed ($P<0.05$) between 0 (control) and 300 µg/g BW. When the females and males were treated with CE-enriched feed at the concentration of 500 and 400 µg/g BW, the broodstocks showed a similar response to eyestalk ablation.

B. Reproductive Performance

CE can promote the development of reproductive organs and contribute to the formation of spermatophores in broodstock held in captivity. Figure 3 illustrates the percentage of matured male broodstock identified by the presence of spermatophores throughout the study period.

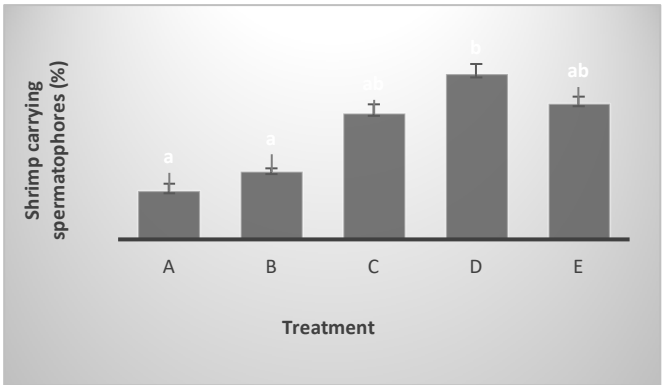

Fig. 3 The number of male broodstock carrying spermatophores after application of CE treatment. A=control/without extract (0 µg/g BW); B=300 µg/g BW, C=400 µg/g BW; D=500 µg/g BW, E=eyestalk ablation; and the values with different letters were significantly different (P<0.05)

In Figure 3, the highest percentage of male broodstock carrying spermatophore was obtained for broodstock fed with CE-enriched feed at the concentration of 500 µg/g BW, which was 85%. The analysis showed no significant difference (P>0.05) between the male broodstock fed with CE-enriched feed at the concentration of 500 and 400 µg/g BW, including eyestalk ablation. In contrast, there was a significant difference (P<0.05) between the male broodstock fed with CE-enriched feed at the concentration of 500 with 0 µg/g BW (control) and 300 µg/g BW. Based on the result, applying CE at the concentration of 500 µg/g BW was the best dose option for supplementing captive male tiger shrimp broodstock because it helped stimulate spermatophore production compared to the other treatments. There were indications that the higher the dose of CE, the mating female tends to increase.

The application of CE at various concentrations has been found to impact the gonad maturity of captive female tiger shrimp broodstock. When female broodstock was treated with CE, the number of matured male broodstock increased, as shown in Figure 4.

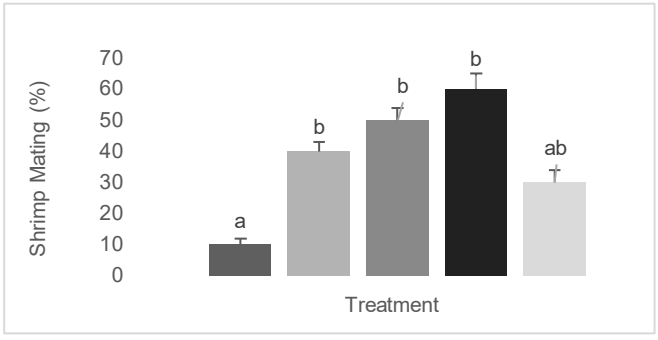

Fig. 4 The percentage of mating female broodstock after application of CE treatment. A=control/without extract (0 µg/g BW); B=300 µg/g BW, C=400 µg/g BW; D=500 µg/g BW, E=eyestalk ablation; and the values with different letters were significantly different (P<0.05)

Among the various concentrations tested, the highest percentage (60%) of matured broodstock was accomplished with the CE treatment at 500 µg/g BW. This result showed a significant difference (P<0.05) from the control treatment, where CE was not administered. However, the 500 µg/g treatment did not exhibit a significant difference (P>0.05) compared to the treatments with CE concentrations of 300 and 400 µg/g BW, as well as the eyestalk ablation treatment. These findings indicate that the response of female tiger shrimp broodstock to CE tends to increase with higher concentrations of CE.

The present study revealed that the CE application positively influences the percentage of mating female broodstock and the development of GML as the concentration of CE in the feed increases. The highest rate of female tiger shrimp reaching GML IV was achieved at a dose of 500 µg/g BW, with a percentage of 62.5% (Table 1). CE and eyestalk ablation could stimulate gonad development up to GML IV, while the control group (without CE) only reaches GML II.

TABLE I
GONADAL MATURITY LEVEL (GML) OF FEMALE BROODSTOCK FED WITH CE-ENRICHED FEED AT DIFFERENT CONCENTRATIONS (N=16)

Treatment	GML				Percentage of matured broodstock (%)
	I	II	III	IV	
A	2	2	0	0	25
B	2	1	1	1	31.25
C	2	2	2	2	50
D	2	2	2	4	62.5
E	2	2	1	1	37.5

Notes: A=control/without extract (0 µg/g BW); B=300 µg/g BW, C=400 µg/g BW; D=500 µg/g BW, E=eyestalk ablation, and (-) = un-spawn female broodstock

The highest number and diameter of eggs were observed in the application of CE at a dose of 500 µg/g BW (337,339 eggs and 289.5 µm, respectively), followed by 400 and 300 µg/g BW as well as eyestalk ablation, respectively (Table 2).

TABLE II
EGG DIAMETER AND NUMBER OF NAUPLI OF FEMALE BROODSTOCK AT DIFFERENT TREATMENTS DURING THE STUDY

Parameters	Treatment				
	A	B	C	D	E
Egg number (pcs)	-	276,570	303,663	337,339	271,550
Egg diameter (µm)	-	276.3	282.6	289.5	264.6
Number of nauplii (ind)	-	6,143	15,057	16,175	13,738

Notes: A=control/without extract (0 µg/g BW); B=300 µg/g BW, C=400 µg/g BW; D=500 µg/g BW, E=eyestalk ablation, and (-) = un-spawn female broodstock

A relatively similar response pattern occurs in egg diameter and number of nauplii. CE treatment in the feed tends to increase the number and diameter of eggs and the number of nauplii produced as indicators of improved reproductive performance in tiger shrimp broodstock. The result of the gene expression of this present study showed that the PCR process had been run usually by indicating the presence of β-actin words in all samples (Figure 5A). The expression of the gametogenesis gene in the male broodstock and the vitellogenin gene in the female broodstock are shown in Figure 5B and Figure 5C, respectively.

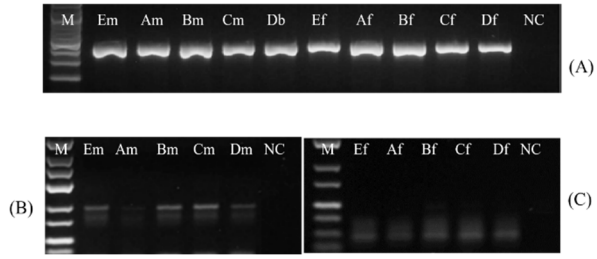

Fig. 5 Expression of β-actin (A), gametogenesis (B), and vitellogenin (C) genes on the tiger shrimp given by different concentrations of CE (A=control/without quote, B=300 µg/g BW, C=400 µg/g BW; D=500 µg/g BW, E=eye stalk ablation, m=male, f=female, and M=DNA marker)

Figure 5 shows that the 400 µg/g BW concentration showed a higher expression for both genes than the other treatments. A higher word was even with the higher extract concentration; however, the gametogenesis expression in males at 500 µg/g BW seemed relatively lower than 400 µg/g BW. In vitellogenin analysis, the higher the concentration of CE, the higher the vitellogenin expression was observed in females. The 400-500 µg/g BW concentration was relatively higher than the control (without extract) and the ablation.

C. Discussion

Feeding captive tiger shrimp with CE-containing feed positively influences the reproductive performance of both male and female broodstock, depending on the given CE concentration. Shrimp mating is generally done at night, after the female shrimp molt. Molting is a natural process observed in various species of invertebrates, such as crustaceans and shrimp[27]. Molting is an indispensable biological process that is key in regulating survival, development, metamorphosis, and reproduction [28]. Molting in female shrimp broodstock has a key role in shrimp reproduction. Molting female is a critical preparatory stage for natural mating. During molting, the female sheds the old exoskeleton [23], and their telycum becomes softer, making it easier for them to receive sperm from male shrimp.

Molting in male shrimp broodstocks is also necessary. Molting in male shrimp broodstock accelerates the formation of spermatophores, and spermatophores formed after molting have qualities similar to those developed gradually [29]. This study showed a difference in the percentage of molting observed in captive female and male tiger shrimp. Female shrimp broodstock given CE at a concentration of 500 µg/g BW gave a molting percentage of up to 40%. Meanwhile, male tiger shrimp broodstock that was also fed CE-containing feed had a molting percentage of about 31% when the CE concentration was 400 µg/g BW. It shows that female shrimp require a higher attention of CE to carry out the molting process than male tiger shrimp.

However, there is an exciting difference in the mature needs of the gonads. When feed contains CE at the same concentration (500 µg/g BW), the percentage of gonadally mature male and female tiger shrimp is significantly higher compared to lower concentration treatment or control treatment and eyestalk ablation. The percentage of gonadally mature male tiger shrimp reaches 80%, while the percentage of gonadally mature female shrimp is around 60%. However, CE administration at higher concentrations (500 and 400 µg/g BW) showed no significant difference ($P>0.05$) in molting percentage and gonadal maturity in both males and females. These two CE concentrations gave a significantly higher percentage of molting and gonadal maturity than the control treatment and eyestalk ablation.

Results indicate that CE administration is more effective in increasing the percentage of molting and gonadal development than eyestalk ablation. When compared to giving CE by injection at a dose of 100 µg/g BW (50%) [1], CE administration through feed with a concentration of 500 µg/g BW resulted in a lower molting percentage of 2.22 times (40%) in female broodstock. It proves that juvenile hormone compounds, such as MF, contained in CE, can affect the molting process and gonad maturity in female and male tiger shrimp broodstock. As reported in different species, MF regulates freshwater crabs' reproduction and molting of *Oziotelphusa senex*. [14]

In the other species, MF effectively increased spermatozoa count and reproductive index in the narrow-clawed crayfish *Pontastacus leptodactylus* [30]. MF at lower concentrations (120 ng/g BW) has significantly improved the spermatophores quality of white shrimp *Litopenaeus vannamei* after five injections during 36 days of treatment [30]. In the injection method, the concentration of CE needed is lower, which may be due to all CE entering the body of shrimp, in contrast to oral administration through feed, which allows not all feed containing CE to be eaten by shrimp. However, the oral administration of CE through feeding mixing is relatively safe and does not cause stress in shrimp. MF can increase shrimp molting by inhibiting Molting Inhibition Hormone (MIH), where MIH inhibition allows organ Y to secrete ecdysones, which initiate and maintain molting [14], [31]. MF regulates physiological processes such as metamorphosis and reproduction in most crustaceans and insects [23].

MF, a chemical structure very similar to the juvenile hormones found in crustaceans, plays a role in the growth, development, and stimulation of insects in reproduction [32]. MF is essential in controlling gonadal development and maturity in some invertebrate organisms, especially crustaceans, such as crabs and shrimps. Inducing this compound can affect hormone metabolism in shrimp broodstock and regulate gonad maturity levels and mating [33].

MF should be administered in appropriate concentrations to achieve the desired effect at the degree of gonadal maturity. The optimal concentration may vary depending on the crustacean species and environmental conditions. In this study, females fed CE-enriched feed at the highest concentration of 500 µg/g BW showed the highest number of broodstock reaching GML IV. Although other factors affect GML, such as the weight and quality of shrimp broodstock, feed with good protein content, and sound environment, hormone manipulation can also trigger gonad maturity[17].

Administration of CE, especially at a dose of 500 µg/g BW, improved the reproductive performance of the tiger shrimp broodstock. The increase in the number and diameter of eggs and the number of nauplii (larvae) served as indicators of improved reproductive performance. These findings showed that CE treatment in the shrimp feed could enhance the reproductive potential of tiger shrimp broodstock. The higher number of eggs indicates a higher reproductive output, which

can benefit commercial shrimp farming. Moreover, the larger diameter of eggs suggests potential improvements in egg quality and viability. Larger eggs may contain more nutrients and energy reserves for the developing embryos, leading to higher survival rates and healthier offspring [34],[35]. MF as a component in CE affects the development of gonads and spermatogenesis. It will undoubtedly affect the acquisition of eggs and spermatophores through injection application and orally in feed [36]. [37].

Additionally, the study mentioned a relative similarity in the response pattern of egg diameter and nauplii quantity. It implies that the positive effects of CE treatment extend beyond just egg production and impact the eggs' subsequent development and hatching success. The increase in nauplii quantity further supports the notion that CE treatment improves reproductive performance by enhancing the overall reproductive potential of the broodstock.

A protein called vitellogenin is essential in the reproductive process of shrimp and other invertebrate animals. The vitellogenin gene controls the production of vitellogenin in the body of female shrimp, which is used in egg formation. Disruptions in vitellogenin gene expression can interfere with egg maturation, reduce the number of eggs produced, and affect egg quality [38]. In addition, spermatogenesis gene expression in male shrimp plays an essential role in sperm production. Disruptions in the face of these genes can reduce sperm production, affect the ability of male shrimp to fertilize eggs, and affect sperm quality [39]. Thus, the vitellogenin and spermatogenesis genes are crucial in the shrimp reproduction process and significantly impact the reproduction and quality of shrimp offspring.

The β-actin gene was employed as an internal control to evaluate the expression of gametogenesis genes in male broodstock and vitellogenin genes in female broodstock. The β-acting gene served as a reference point to validate successful gene isolation, ensuring that the samples were free from contamination. By utilizing the β-acting gene as an internal control, any variations in gene expression observed could be attributed to the specific genes under investigation rather than unintended factors such as contamination or technical errors. This control was crucial for accurately assessing the expression patterns of the target genes and obtaining reliable results in the study of gametogenesis and vitellogenin gene expression in the respective broodstock [26]. The development of female tiger shrimp growth could be determined by vitellogenesis gene expression.

Vitellogenesis is a principal process during ovarian maturation in crustaceans [40]. Based on the findings presented in Figure 4, it is evident that the concentration of 400 µg/g BW led to elevated expression levels of both the gametogenesis and vitellogenin genes, surpassing the expression levels observed in the other treatments. It indicates that the concentration above may positively influence the expression of these genes. It is worth noting that a higher concentration of the compound being studied (CE) exhibited a direct correlation with increased gene expression. The results obtained from the analysis of gametogenesis-related gene expression in male tiger shrimp *P. monodon* indicated that the induction of 17α-methyltestosterone hormone 1-2 times resulted in higher expression levels (2.5-2.75 times) compared to the other treatment conditions [41].

However, in the case of gametogenesis gene expression in males, the concentration of 500 µg/g BW appeared to have a relatively lower expression than the 400 µg/g BW concentration. This observation suggests that there might be a concentration-dependent effect on gametogenesis gene expression, where an excessively high concentration (500 µg/g BW) may have a diminishing impact. In the analysis of vitellogenin expression, it was observed that higher concentrations of the extract (CE) corresponded to increased vitellogenin expression in females. Specifically, 400-500 µg/g BW concentrations exhibited relatively higher vitellogenin expression than the control group (without extract) and the ablation group. This finding suggests that the extract at these concentrations has induced vitellogenin ovarian maturation, indicated by increased vitellogenin gene expression in female broodstock[42].

The detailed mechanism of Gonad-inhibiting hormone (GIH) regulating vitellogenin expression is still ambiguous. However, Kluebsoongnoen et al. [40],[43] stated that GIH, a neuronal peptide hormone from the eyestalks of tiger shrimp, negatively regulated the vitellogenin process. A study of the white shrimp *Litopenaeus vannamei* revealed two stages of vitellogenin gene expression for the completion of vitellogenesis: the extra-ovarian hepatopancreatic stage followed by the intraovarian stage performed by the ovary [44]. They also state that shrimp's vitellogenin genes will likely regulate growth and the molt cycle. MF induces the synthesis of vitellogenin in crustacea either directly or indirectly ripening the ovaries[14], [15]. Overall, the results indicate that the concentration of CE plays a crucial role in regulating the expression of the gametogenesis and vitellogenin genes. The 400 µg/g BW concentration appears to be particularly effective in enhancing both gene expressions.

IV. Conclusion

Administrating *Cyperus* sp extract containing MF compound can improve the reproductive breeding of captive tiger shrimp. The dosage between 400-500 µg/g body weight in the diet showed positive results of increased molting, more males carrying with spermatophores, successful mating, and better egg production and quality. These findings recommended using the optimal dosage of Cyperus extract at 400-500 µg/g body weight in the diet of tiger shrimp broodstock.

Acknowledgment

The authors thank the National Research and Innovation Agency (BRIN), the Research Institute for Brackish Water Aquaculture and Fisheries Extension (RIBAFE), and the Indonesia Endowment Fund for Education (LPDP) of the Ministry of Finance of Indonesia through the RIIM Program Phase 2 for supporting this research. The authors also thank technicians at the Biotechnology Laboratory of RIBAFE and all those who assisted in supporting this study.

References

[1] M. Eko Falah, E. H. Hardi, H. Pagoray, G. Saptiani, and A. Agustina, "Performance of Windu Shrimp (Penaeus monodon) Cultivated in a Silvofishery Pond with the Application of Phytoimmune®," Aquac. Indones., vol. 24, no. 2, pp. 61–66, 2024, doi: 10.21534/ai.v24i2.19.

[2] Darryl Jory, “Annual farmed shrimp production survey: A slight decrease in production reduction in 2023 with hopes for renewed growth in 2024,” Glob. Self. Alliance, no. October 2023, pp. 1–8, 2023, [Online]. Available: https://www.globalseafood. org /an advocate/ annual-farmed-shrimp-production-survey-a-slight-decrease-in- production-reduction-in-2023-with-hopes-for-renewed-growth-in-2024/

[3] L. L. Wong et al., “Genetic diversity and population structure of wild and domesticated black tiger shrimp (Penaeus monodon) broodstocks in the Indo-Pacific regions using consolidated mtDNA and microsatellite markers,” Gene Reports, vol. 23, no. January, p. 101047, 2021, doi: 10.1016/j.genrep.2021.101047.

[4] S. S. Santander-Avanceña, “Enhancing the Reproductive Performance of Cultured Shrimp: novel information on scent, maturation, and mating,” Fish People, vol. 20, no. 1, pp. 13–19, 2022.

[5] A. F. Montoya-López, A. M. Tarazona-Morales, M. Olivera-Angel, and J. J. Betancur-López, “Genetic diversity of four broodstocks of tilapia (Oreochromis sp.) from Antioquia, Colombia,” Rev. Colomb. Ciencias Pecu., vol. 32, no. 3, pp. 201–213, 2019, doi:10.17533/udea.rccp.v32n3a05.

[6] R. Rosmiati et al., “Improved reproductive performance of captive male broodstock of tiger shrimp Penaeus monodon with 17α-methyltestosterone induction,” Isr. J. Aquac. - Bamidgeh, vol. 74, 2022, doi: 10.46989/001c.31616.

[7] A. Laining, S. Lante, and U. Usman, “Induksi Pematangan Gonad dan Peningkatan Tingkat Pembuahan Telur Induk Udang windu P, Penaeus monodon Melalui rangsangan hormonal Tanpa ablasi mata,” J. Ris. Akuakultur, vol. 10, no. 1, p. 61, 2015, doi:10.15578/jra.10.1.2015.61-68.

[8] K. Sittikankaew et al., “Author Correction: Transcriptome analyses reveal the synergistic effects of feeding and eyestalk ablation on ovarian maturation in black tiger shrimp (Scientific Reports, (2020), 10, 1, (3239), 10.1038/s41598-020-60192-2),” Sci. Rep., vol. 10, no. 1, 2020, doi: 10.1038/s41598-020-62221-6.

[9] I. Nur, H. Iyen, and Y. Yusnaini, “The Effect of Eyestalk Ablation on Several Immunologic Variables in Litopenaeus vannamei,” J. Aquac. Fish Heal., vol. 10, no. 1, p. 34, 2021, doi: 10.20473/jafh.v10i1.18926.

[10] W. Wen, X. Chen, W. Zhao, Q. Yang, J. G. Qin, and Z. Ma, “Evaluation of reproductive capacity in pond-reared Penaeus monodon after unilateral eyestalk ablation,” Pak. J. Zool., vol. 52, no. 4, pp. 1283–1289, 2020, doi: 10.17582/journal.pjz/20190518040508.

[11] J. E. Moreno-Reyes, M. C. Morales, and J. A. Meruane, “A feasible path towards year-round production: Effects of temperature and photoperiod on ovarian maturity of subtropical palaemonid, the river shrimp, Cryphiops caementarius,” Aquac. Reports, vol. 21, no. January, p. 100809, 2021, doi: 10.1016/j.aqrep.2021.100809.

[12] X. Meng, M. Zhang, B. Gao, J. Lv, J. Li, and P. Liu, “Integrative Proteomic and MicroRNA Analysis: Insights Into Mechanisms of Eyestalk Ablation-Induced Ovarian Maturation in the Swimming Crab Portunus trituberculatus,” Front. Endocrinol. (Lausanne)., vol. 11, no. August, pp. 1–14, 2020, doi: 10.3389/fendo.2020.00533.

[13] S. Jia et al., “Molecular Characterization Related to Ovary Early Development Mechanisms after Eyestalk Ablation in Exopalaemon carinicauda,” *Biology (Basel).*, vol. 12, no. 4, 2023, doi:10.3390/biology12040596.

[14] P. R. Reddy and M. Arifullah, “Dietary methyl farnesoate, a potential growth inducer in male crab Oziothelphusa senex senex,” IOP Conf. Ser. Earth Environ. Sci., vol. 756, no. 1, 2021, doi: 10.1088/1755-1315/756/1/012062.

[15] B. S. Reddy and P. R. Reddy, “Methyl farnesoate induced ovarian vitellogenin synthesis in freshwater rice field crab Oziothelphusa senex senex Fabricius.,” J. Infertil. Reprod. Biol., vol. 3, no. 4, pp. 237–244, 2015.

[16] C. Yao, Y. Sun, Z. Zhang, X. Jia, P. Zou, and Y. Wang, “Integration of RNAi and RNA-seq uncovers the regulation mechanism of DDX20 on vitellogenin expression in Scylla paramamosain,” Comp. Biochem. Physiol. - Part D Genomics Proteomics, vol. 44, no. October, p. 101028, 2022, doi: 10.1016/j.cbd.2022.101028.

[17] B. Almazrouei et al., “Steroid hormones in wastewater: Sources, treatments, environmental risks, and regulations,” Emerg. Contam., vol. 9, no. 100210, pp. 1–12, Jun. 2023, doi:10.1016/j.emcon.2023.100210.

[18] Y. Taheri et al., “Cyperus spp.: A Review on Phytochemical Composition, Biological Activity, and Health-Promoting Effects,” Oxid. Med. Cell. Longev., vol. 2021, 2021, doi:10.1155/2021/4014867.

[19] S. B. Babiaka, A. F. A. Moumbock, S. Günther, and F. Ntie-Kang, “Natural products in Cyperus rotundus L. (Cyperaceae): An update of the chemistry and pharmacological activities,” RSC Adv., vol. 11, no. 25, pp. 15060–15077, 2021, doi: 10.1039/d1ra00478f.

[20] E. Suryati, S. Lante, A. Tenriulo, Rosmiati, and A. Parenrengi, “Potential of methyl fernesoate isolate from the Cyprus extract in prospective broodstock of shrimp (Penaeus monodon. Fab),” in IOP Conference Series: Earth and Environmental Science, 2021, vol. 860, no. 1. doi: 10.1088/1755-1315/860/1/012038.

[21] R. and A. P. Suryati. E, Samuel Lante, A Tenriulo, “Potential of methyl fernesoate isolate from the Cyprus extract in prospective broodstock of shrimp (Penaeus monodon. Fab),” IOP Conf. Ser. Earth Environ. Sci. 860 012038, vol. 860, no. 1, 2021, doi: 10.1088/1755-1315/860/1/011001.

[22] M. A. B. Siddik, M. J. Foysal, R. Fotedar, D. S. Francis, and S. K. Gupta, “Probiotic yeast Saccharomyces cerevisiae coupled with Lactobacillus casei modulates physiological performance and promotes gut microbiota in juvenile barramundi, Lates calcarifer,” Aquaculture, vol. 546, no. March 2021, p. 737346, 2021, doi:10.1016/j.aquaculture.2021.737346.

[23] W. Promwikorn, P. Kirirat, and P. Thaweethamsewee, “Index of molt staging in the black tiger shrimp (Penaeus monodon),” J. Sci. Technol., vol. 26, no. 5, pp. 765–772, 2004.

[24] B. C. Paran, I. J. Fierro, and B. Tsukimura, “Stimulation of ovarian growth by methyl farnesoate and eyestalk ablation in penaeoidean model shrimp, Sicyonia ingentis Burkenroad, 1938,” Aquac. Res., vol. 41, no. 12, pp. 1887–1897, 2010, doi: 10.1111/j.1365-2109.2010.02612.x.

[25] S. H. Kwan Tiu, J. Benzie, and S. M. Chan, “From hepatopancreas to ovary: Molecular characterization of a shrimp vitellogenin receptor involved in the processing of vitellogenin,” Biol. Reprod., vol. 79, no. 1, pp. 66–74, 2008, doi: 10.1095/biolreprod.107.066258.

[26] A. Parenrengi, A. Tenriulo, A. Alimuddin, and S. Sukenda, “Enhancement of Tiger Shrimp Penaeus monodon Resistance to White Spot Syndrome Virus by Overexpression of Antiviral Gene,” Int. J. Agric. Biol., vol. 25, no. 2, pp. 277–284, 2021, doi:10.17957/IJAB/15.1667.

[27] N. Hosamani, S. Reddy B, and R. Reddy P, “Crustacean Molting: Regulation and Effects of Environmental Toxicants,” J. Mar. Sci. Res. Dev., vol. 07, no. 05, 2017, doi: 10.4172/2155-9910.1000236.

[28] K. Toyota et al., “Eyestalk transcriptome and methyl farnesoate titers provide insight into the physiological changes in the male snow crab, Chionoecetes opilio, after its terminal molt,” Sci. Rep., vol. 13, no. 1, pp. 1–9, 2023, doi: 10.1038/s41598-023-34159-y.

[29] A. Braga, D. L. A. Lopes, L. H. Poersch, and W. Wasielesky, “Spermatophore replacement of pink shrimp Farfantepenaeus brasiliensis after manual extrusion: Effect of molting,” Aquaculture, vol. 433, pp. 313–317, 2014, doi: 10.1016/j.aquaculture.2014.06.032.

[30] J. Alfaro, G. Zúñga, A. García, and E. Rojas, “Preliminary evaluation of the effect of Juvenile hormone III and methyl farnesoate on spermatophore quality of the white shrimp, Litopenaeus vannamei Boone, 1931 (Decapoda: Penaeidae),” Rev. Biol. Mar. Oceanogr., vol. 43, no. 1, pp. 167–171, 2008, doi: 10.4067/S0718-19572008000100018.

[31] S. Techa and J. S. Chung, “Ecdysteroids regulate the levels of molt-inhibiting hormone (MIH) expression in the blue crab, Callinectes sapidus,” PLoS One, vol. 10, no. 4, pp. 1–19, 2015, doi:10.1371/journal.pone.0117278.

[32] E. Wynants et al., “Uncovering the role of juvenile hormone in ovary development and egg laying in bumble bees,” J. Insect Physiol., vol. 150, no. August, 2023, doi: 10.1016/j.jinsphys.2023.104557.

[33] M. Bal and A. G. Harlıoğlu, “Effects of methyl farnesoate injection on spermatozoa number and reproductive indices in the narrow-clawed crayfish Pontastacus leptodactylus,” Nauplius, vol. 30, 2022, doi:10.1590/2358-2936e2022006.

[34] A. Ciji et al., “Comparative assessment of egg and larval quality traits of progeny from wild-collected and captive-matured brooders of endangered golden mahseer, Tor putitora: A prelude to quality broodstock development and seed production,” Aquaculture, vol. 552, no. August 2021, p. 737949, 2022, doi:10.1016/j.aquaculture.2022.737949.

[35] H. Jeuthe, E. Brännäs, and J. Nilsson, “Effects of egg size, maternal age and temperature on the egg, viability of farmed Arctic charr,” Aquaculture, vol. 408–409, pp. 70–77, 2013, doi:10.1016/j.aquaculture.2013.05.034.

[36] B. Tsukimura, “Crustacean vitellogenesis: its role in oocyte development1,” Am. Zool., vol. 41, no. 3, pp. 465–476, 2001, doi:10.1668/0003-1569(2001)041[0465:CVIRIO]2.0.CO;2.

[37] J. Alfaro and A. a Vaca, “Ovarian maturation and spawning in the white shrimp, Penaeus vannamei, by serotonin injection,” Aquaculture, vol. 182, no. 3–4, pp. 373–385, 2000.
[38] W. S. Tsang, L. S. Quackenbush, B. K. C. Chow, S. H. K. Tiu, J. G. He, and S. M. Chan, “Organization of the shrimp vitellogenin gene: Evidence of multiple genes and tissue-specific expression by the ovary and hepatopancreas,” Gene, vol. 303, no. 1–2, pp. 99–109, 2003, doi:10.1016/S0378-1119(02)01139-3.
[39] U. Saetan and W. Chotigeat, “Differentially expressed genes in the testes from early to mature development of banana shrimp (Fenneropenaeus merguiensis),” PLoS One, vol. 18, no. 10 October, pp. 1–15, 2023, doi: 10.1371/journal.pone.0292127.
[40] J. Kluebsoongnoen, S. Panyim, T. J. Sarnowski, and A. Udomkit, “Retinoid X receptor modulates vitellogenin gene expression in black tiger shrimp, Penaeus monodon,” Comp. Biochem. Physiol. -Part A Mol. Integr. Physiol., vol. 254, p. 110877, 2021, doi:10.1016/j.cbpa.2020.110877.
[41] R. Rosmiati et al., “Application of 17α-methyltestosterone to enhance broodstock maturation of the domesticated black tiger shrimp (Penaeus monodon): A comparative study of injection method and oral method,” in IOP Conference Series: Earth and Environmental Science, 2022, vol. 1119, no. 1. doi: 10.1088/1755-1315/1119/1/012038.
[42] A. A. Raghavan, “Effect of Methyl Farnesoate Administration on Ovarian Growth and Maturation in the Freshwater Crab Travancoriana schirnerae,” J. Aquat. Biol. Fish., vol. 22, no. 5, pp. 257–271, 2018, [Online]., vol. 22, no. 5, pp. 257–271, 2018.
[43] J. Kluebsoongnoen, S. Panyim, and A. Udomkit, “Regulation of vitellogenin gene expression under the negative modulator, gonad-inhibiting hormone in Penaeus monodon,” Comp. Biochem. Physiol. - Part A Mol. Integr. Physiol., vol. 243, no. February, p. 110682, 2020, doi: 10.1016/j.cbpa.2020.110682.
[44] W. Wang et al., “Investigation of Gene Sequence Divergence, Expression Dynamics, and Endocrine Regulation of the Vitellogenin Gene Family in the Whiteleg Shrimp Litopenaeus vannamei,” Front. Endocrinol. (Lausanne)., vol. 11, no. November, pp. 1–15, 2020, doi:10.3389/fendo.2020.577745.