The potential of banana hump extract (*Musa acuminata*) in prospective tiger shrimp (*Penaeus monodon* Fab) broodstock

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Abstract. *Musa acuminata* is one of the plants that contain serotonin, a neurotransmitter compound that plays a role in the reproductive process in crustaceans and is used to spur the development of reproductive organs in tiger shrimp (Penaeus monodon. Fab) broodstock candidates. In this research, serotonin isolation from the hump of *M. acuminata* was carried out through extraction techniques, maceration, and purification using acetone and alcohol. The serotonin isolate obtained was tested for purity by HPLC, followed by measurement of UV and IR spectra. Banana hump extract was applied to tiger shrimp through injection with concentrations of 0, 25, 50, and 75 ug g^{-1} body weight (BW), commercial serotonin, and eyestalk ablation as a positive control. Observations made included the level of gonadal maturity using PCR technique with Vittelogenin primers for females and LVdmc for males, molting frequency, histology, and total hemocytes. The analysis showed the highest survival in the treatment of BW 50 and 75 ug g^{-1}, which was also better than ablation and commercial serotonin for gonad development and molting frequency. Thus, the results obtained are expected to improve the quality of tiger shrimp broodstock candidates in the right amount and time as needed with good quality.
Key Words: broodstock candidate, gonad development, serotonin.

Introduction. Tiger shrimp (*Penaeus monodon*) domestication is one of the Indonesian government's programs to properly control and trace its survival and reproductive system as a sustainable and environmentally friendly conservation effort. The problem in obtaining shrimp broodstock is that adult shrimp are immature, avoiding mating (Lante et al 2018). One way to trigger gonadal maturity in tiger shrimp is by eye stalk ablation (Nur et al 2021) but this technique can cause egg rot, decreased immunity in shrimp mothers, and reduced quality and quantity of larvae over time, which makes them susceptible to disease and unable to reproduce again (Tsukimura 2001; Aktaş & Kumlu 2005).

Quality fry plays an important role in improving spermatophore quality and facilitating the maturation of tiger shrimp broodstock gonads from ponds through proper hormone induction.

Serotonin has been used for shrimp broodstock injection (Wongprasert et al 2006). In *Penaeus vannamei*, serotonin affects broodstock maturity and spawning by playing an important role as a neurotransmitter in the reproductive process (Vaca & Alfaro 2000). In plants, serotonin is often found in bananas, potatoes, kiwi, and lemon bacilli, widely applied for human medicine, mainly to overcome stress and emotional disorders (Ramakrishna & Ravishankar 2011; Erland et al 2018). The extraction and use of serotonin from herbal ingredients have also been pioneered and carried out through screening, among others, from banana humps, shrimp waste extracts, shellfish, and marine worms (Nagur-Babu et al 2013; Erland et al 2018; Gonçalves et al 2022).

Results from previous studies illustrate the positive response of tiger shrimp broodstock to serotonin administration at a bodyweight concentration of 50 µg g^{-1}, which

demonstrated the ability to spur gonadal maturity and produce larvae (Wongprasert et al 2006). However, a more detailed analysis of the optimal dosage and method of administration of serotonin to the broodstock is still necessary. Therefore, the purpose of this study was to determine the technique of administering serotonin from banana hump isolate to improve the quality of spermatophores in male shrimp and spur gonad maturity in female shrimp to ultimately increase the production of domesticated tiger shrimp.

Material and Method

Provision of plants. The hump of the banana (*Musa acuminata*) was obtained from the area around Maros. The hump of the fresh bananas was cleaned of adhering dirt, then thinly sliced using a knife, and dried.

Serotonin isolation. Isolation of serotonin from banana humps was carried out by precipitation of serotonin compounds from hot solutions using acetone. Serotonin purification was carried out by recrystallization using a mixture of acetone and alcohol to produce pure crystals with characteristics that are according to serotonin standards (Ly et al 2008). The hump of a dried banana weighed less than 500 g. It was mashed using a blender, placed in a 2 L goblet glass, and 1 L of aquadest was added. The mix was heated until boiling. The pulp was separated from the solution using a sieve with pressure until an aqueous solution was obtained. From the hot solution obtained, approximately 1/7 was mixed with 1 L of acetone at a temperature of 80^{o}C. After mixing, the rest of the solution was added until it precipitated. The mixture was cooled at room temperature for one night, then the formed precipitate was separated by centrifugation. The formed precipitate was washed with 50% acetone. The filtrate was evaporated using a rotary evaporator at temperatures below 50^{o}C. The precipitate obtained from evaporation was dissolved with 55 mL of water, and heated until boiling. 350 mL of acetone was added, and the mix was kept in a cool room for two days. The obtained precipitate was separated by a centrifuge to produce a meat-colored residue and contained 200 units of crude serotonin extract per mg. The remaining filtration was evaporated until dry. 50 mL of 50% methanol was added and dried again. Then, 10 mL of absolute methanol was added. The supernatant was decanted, and the crystals formed were washed with methanol and acetone until white crystals formed (Ramakrishna et al 2011). Serotonin was purified by recrystallization using acetone and solvent alcohol to produce crystals. The purity test of the compound obtained was carried out using the Thermo brand HPLC. The melting point test was carried out using the WRS-200 melting point apparatus. The UV spectrum measurement was carried out using the Variant UV_VIS spectrophotometry, while the IR spectrum measurement was carried out using a Shimadzu spectrophotometer, type IRP-21.

The application of serotonin isolates from the banana hump in tiger shrimp. This research was conducted at a tiger shrimp hatchery, in Barru Regency, South Sulawesi, Indonesia. The treatment trials were: (1) application of serotonin from banana humps to male and female tiger shrimps; (2) eye ablation without hormones and serotonin induction as a positive control and injection with saline solution as a negative control. The hormone used in this study is serotonin produced from banana humps through isolation, referring to Erland et al (2018), while the commercial serotonin standard is a product of Sigma Cat: H 7752. Before use, serotonin was dissolved in saline solution as a stock solution with a concentration of 20 mg mL^{-1}. Serotonin hormone injection was done once a week three times, with doses of 25 µg g^{-1} body weight (BW), 50 µg g^{-1} BW, and 75 µg g^{-1} BW shrimp broodstock, plus ablation as positive control and saline solution as a negative control. The dose administered refers to previous research (Aktaş & Kumlu 2005; Wongprasert et al 2006).

The tiger shrimp broodstock candidates used in this study were collected from shrimp ponds in Takalar District, South Sulawesi. The study was conducted with 5 treatments and 3 replicates at a density of 8 tiger shrimp per tank with a volume of 1

tonne. Before use, tiger shrimp were acclimatized for one week. The application was done through injection with commercial serotonin and serotonin isolate extracted from the banana hump, with concentrations of 0, 25, 50, and 75 μg g^{-1} BW and eye stalk ablation (Table 1).

Table 1

Weights and lengths of tiger shrimp before the application of serotonin from banana humps, commercial serotonin, negative control with saline solution, and ablation of the eyelid

Variable	*Application of serotonin from banana humps*	*Commercial serotonin*	*Saline solution*	*Ablation*
Weight of female	90.8±4.25	87.86±7.5	85.75 ±2.1	78.13 ±1.85
Length of female	20±0.08	20.06±0.07	19.66 ± 0.41	19.23 ±0.46
Weight of male	64.4±1.6	52.3±3.67	52.06±3.6	52.30± 2.26
Length of male	18.8±0.3	17.36±0.28	17.2 ± 0.51	16.5± 0.60

The maintenance of brood shrimp was carried out in concrete baths with a volume of 1 ton equipped with aeration and running water. The stock was fed fresh feed including squid, clams, and sea worms, alternately, three times a day at 15% of body weight. The environmental parameters maintained during this study include temperature, salinity, dissolved oxygen (DO), and pH. The temperature during the study ranged from 27 to 34°C, and salinity from 34.2 to 35.5 ppt. The DO levels ranged between 3.3-5.18 mg L^{-1}, and the pH range was between 7.47 and 7.79.

Observed parameters. The survival rate (SR) was observed daily after injection application. The molting frequency was observed daily during maintenance after injection application. The degree of maturity of the gonads was observed in each shrimp visually, but also by PCR, by using hemolymph samples from shrimps.

Total isolation of RNA and synthesis of cDNA. 0.1 mL of hemolymph was collected from the 2nd abdominal segment using a 1.0 mL syringe attached to a measuring needle 26 with a 3.8% sodium citrate anticoagulant (Van-De Braak et al 2002). Hemolymph was centrifuged at a speed of 10000 rpm at a temperature of 4ºC for 5 minutes. The obtained hemocyte pellets were washed once with a cold anticoagulant solution and re-centrifuged at the same rate. Furthermore, total RNA extraction was carried out using a lysis RNA extraction solution with the IQ-2000 method. The synthesis of cDNA (complementary DNA) was carried out using the SensiFast cDNA Synthesis kit (Bioline) by existing procedures. The reaction volume was 20 μL consisting of 4 μL master mix, 1 uL reverse transcript, and 20 μg total RNA. The solution in the microtubes was incubated successively at 25°C for 5 minutes, 42°C (15 minutes), and 85°C for 5 minutes. The cDNA solution was then placed in ice to stop the synthesis reaction and stored at -20°C for further analysis.

Analysis of gene expression associated with tiger shrimp vitellogenesis - PCR. Analysis of the treatment response to vitellogenesis was carried out by taking tiger shrimp hemolymph. Profile analysis of vitellogenesis status was carried out using the quantitative gene transcription expression method with PCR Applied Biosystem Type 2720 Thermal Cycler. Specific primary nucleotide arrangements for expressions encoding vitellogenin (Vg) were: (F)-5', ATT, CGG, AAC, GTG, CAT, TTG, CTG, CA-3' and R-5', GTT, CTC, AAG CAT, TGT, GAC, AGG, ATT-3'. Internal control was carried out using EF-1. PCR analysis using the ABI System Type 2720 Thermal Cycler detection is sequential with 5x Hot Firepol Evagreen PCR mix (ROX). The reaction volume for cDNA amplification was 20 μL with a final concentration of 1x hot Master mix (Rox), F/R primer pairs of 10 pmol of 250 nM each, NFW (nuclease-free water) added up to a volume of 20 μL and cDNA

(0.01 ng uL^{-1}). Cycling temperature conditions for PCR consisted of a holding temperature of 50ºC for 20 minutes, initial denaturation temperature of 95ºC (15 minutes) followed by 95ºC (15 seconds) and annealing temperature of 60ºC (30 seconds) for 40 cycles, and final extension temperature of 72ºC for 20 seconds (Kwan Tiu et al 2008).

Analysis of gene expression associated with spermatogenin (LVDmc) in male tiger shrimp - PCR. Analysis of the treatment response to spermatogenesis was carried out by taking tiger shrimp hemolymph. The analysis of spermatogenesis status was conducted using a quantitative gene transcription expression method via PCR, employing the Applied Biosystems Type 2720 Thermal Cycler. A specific nucleotide primer arrangement for the expression encoding spermatogenic (LVDmc) was used: (F)-5', AGG, CGA, CGG, GTT, TCG, TGA, CA-3' and R-5', TGA, TCT, TCT, GCA, GTC, GTG, ACA, T-3'. Internal control was established using EF-1 for PCR analysis with the ABI System Type 2720 Thermal Cycler. Detection was performed sequentially using a 5x Hot Firepol Evagreen PCR mix (ROX). The reaction volume for cDNA amplification included 20 µL with a final concentration of 1x hot Master mix (Rox), F/R primer pairs of 10 pmol of 250 nM each, NFW (nuclease-free water) was added to obtain a volume of 20 µl, and cDNA (0.01 ng uL^{-1}). Cycling temperature conditions for PCR consist of a holding temperature of 50ºC for 20 minutes, initial denaturation temperature of 95ºC (15 minutes) followed by 95ºC (15 seconds) and annealing temperature of 60ºC (30 seconds) for 40 cycles, and final extension temperature of 72ºC for 20 seconds (Kwan Tiu et al 2008).

Total hemocyte count (THC). THC was determined by taking 0.1 mL hemolymph from the 2nd abdominal segment using a 1 mL syringe attached to a 26-gauge needle with a 3.8% sodium citrate anticoagulant. It was gently moved by homogenization. After discarding the first drop, the second drop was dripped onto the hemocytometer. The total number of hemocyte cells was calculated under a microscope at 100x magnification and estimated after Van De Braak et al (2002).

Results

Isolation of serotonin from banana hump. A crude serotonin extract was obtained through maceration and purification with alcohol, yielding 7% of the dry weight of the raw material. The results of the purity test using HPLC can be seen in Figure 1. Purity test results using HPLC at a wavelength of 225 nm using the isocratic method showed a chromatogram with a single peak at a retention time of 2.167 minutes. Figure 2 presents ultraviolet isolate serotonin spectrum measurement results using UV-Vis.

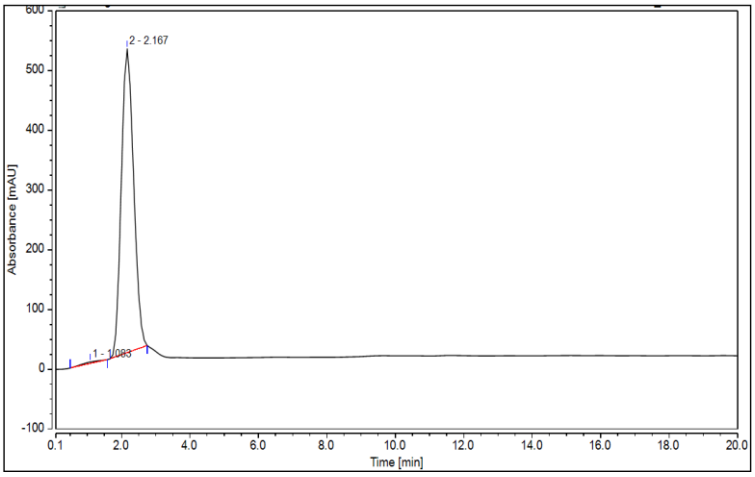

Figure 1. Serotonin chromatogram isolated from the banana hump.

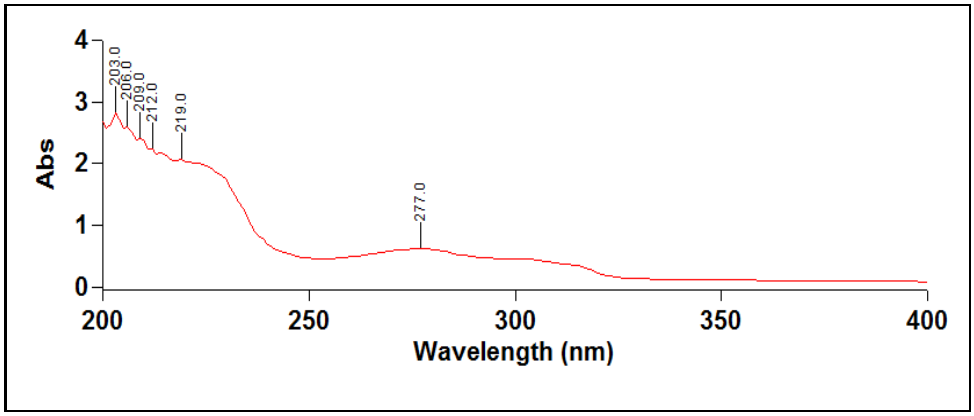

Figure 2. The ultraviolet spectrum of serotonin is isolated from the banana hump.

The spectrum of IR isolates serotonin from the banana hump and the IR serotonin standard can be seen in Figure 3.

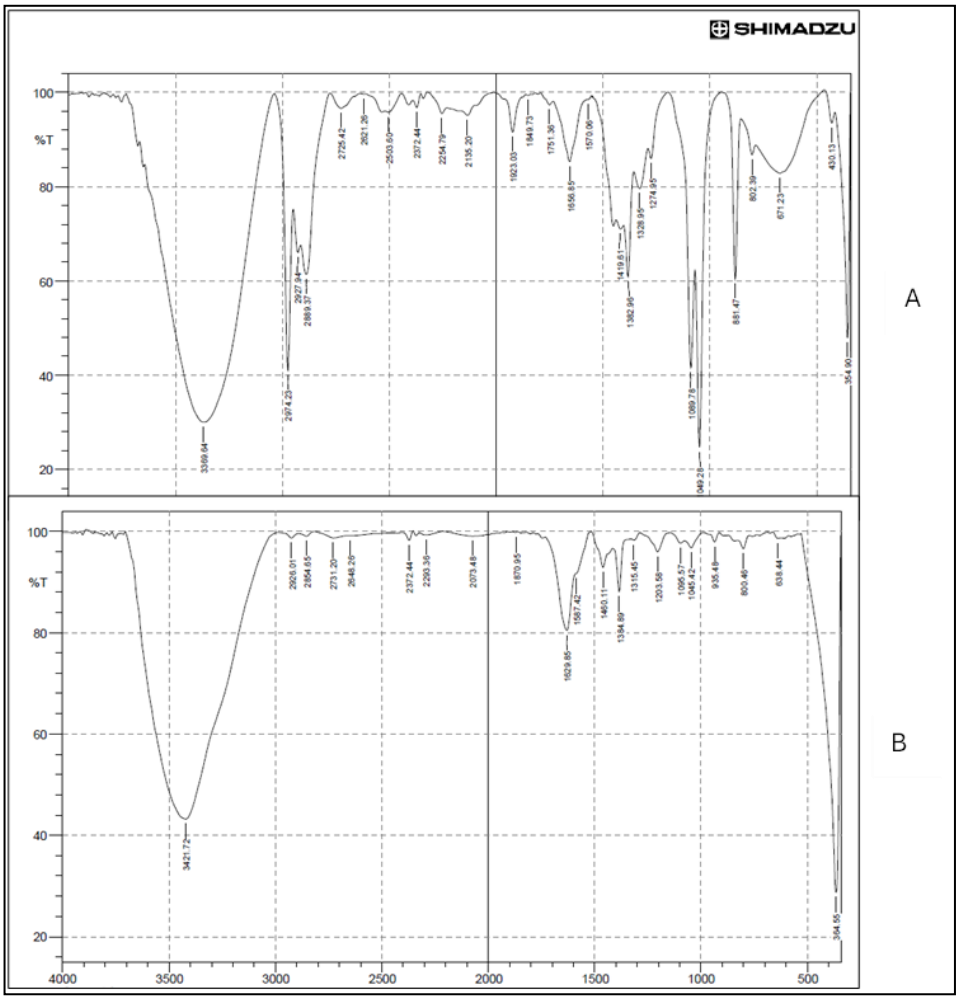

Figure 3. Serotonin infra-red spectra: (A) standard serotonin; (B) isolated from banana hump.

The results of infrared spectrum measurements show the presence of functional groups, including a wide strain at wavelengths 3400-3650 cm^{-1}, indicating the presence of OH groups, then strains at a wavelength of 3300-3500 cm^{-1}, indicating the presence of NH

(amines) groups, and C=C groups at wavelengths 1640-1680 cm^{-1}. This corresponds to the functional group possessed by serotonin compounds.

Melting point. The results of the melting point measurement of serotonin isolated from the banana hump had a value of 167°C.

Serotonin application from the banana hump in tiger shrimp broodstock. Survival of male and female broodstock after application of serotonin from a banana hump (Figure 4).

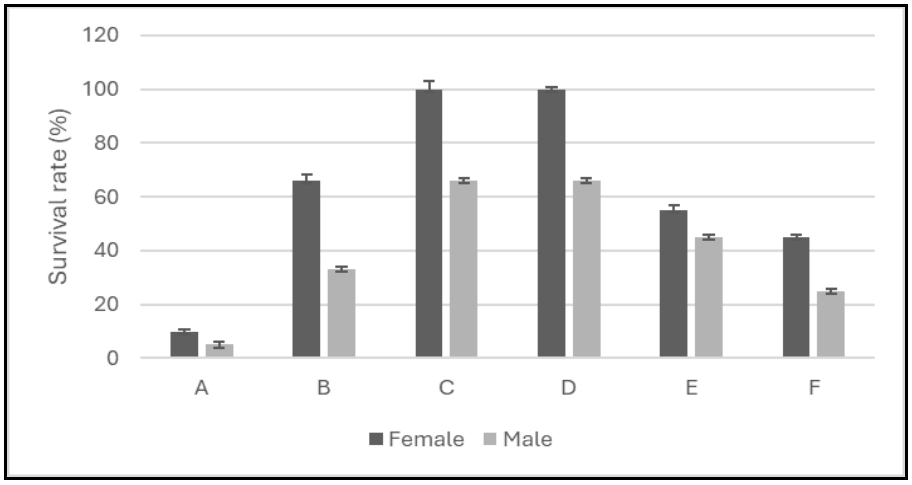

Figure 4. Survival of tiger prawn (*Penaeus monodon*) with the treatment of serotonin isolate from the banana humps, commercial serotonin, and eye stalk ablation; A - saline solution (negative control); B - 25 ug g^{-1} body weight; C - 50 ug g^{-1} body weight; D - 75 ug g^{-1} body weight; E-commercial serotonin; F - ablation of the eye stalks.

Molting frequency. Molting in shrimp broodstock after applying banana extract at different doses is presented in Figure 5.

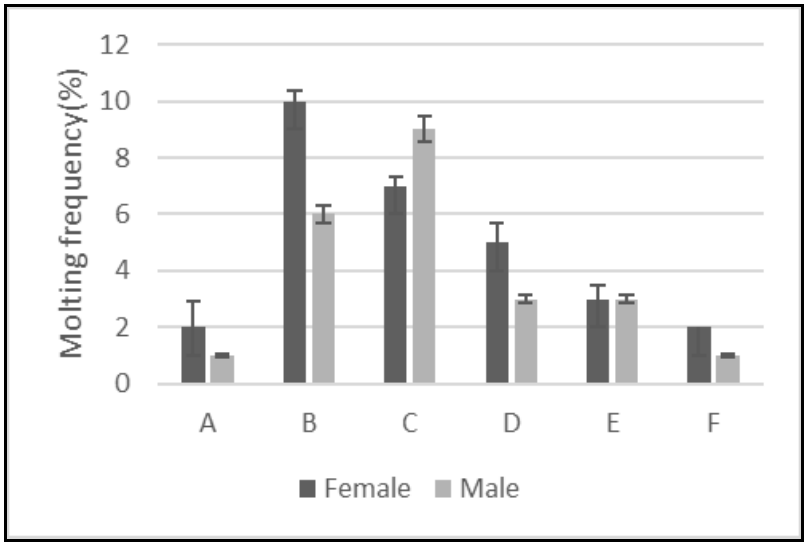

Figure 5. Molting frequency of tiger shrimp (*Penaeus monodon*) broodstock with the treatment of serotonin isolated from the banana hump, commercial serotonin, and eye stalk ablation; A - saline solution (negative control); B - 25 ug g^{-1} body weight; C - 50 ug g^{-1} body weight; D - 75 ug g^{-1} body weight; E-commercial serotonin; F - ablation of the eyes stalk.

In females, the highest molting frequency is at 25 ug g^{-1} BW serotonin; in males, the highest molting frequency was at 50 ug g^{-1} BW serotonin. Negative control and ablation showed low frequency in both male and female shrimp.

Development of gonad. Analysis of vitellogenin development in female tiger shrimp using PCR technique using vitellogenin primers for female tiger shrimp broodstock is presented in Figure 6.

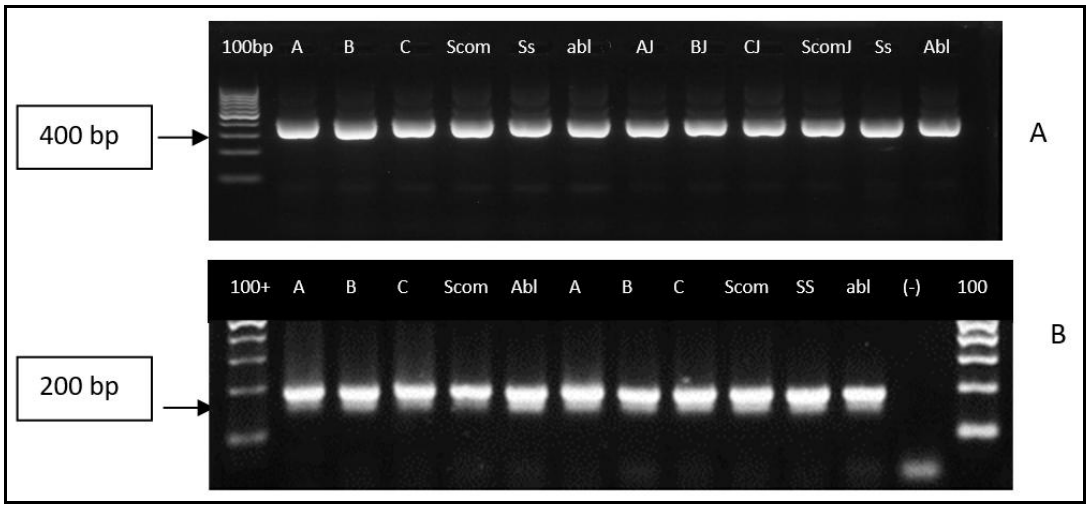

Figure 6 Results of vitellogenin analysis on hemolymph female parent *Penaeus monodon* using β-actin as an internal control (A); and primary vitellogenin (B); A - 25 µg g^{-1} body weight; B - 50 µg g^{-1} body weight; C - 75 µg g^{-1} body weight; Scom - commercial serotonin; Ss - saline solution (control); Abl - ablation.

The level of gonadal maturity in tiger shrimp broodstock after application of banana extract was different. At a dose of 25 µg g^{-1}, the gonadal maturity was up to level IV, in the treatment of 50 µg g^{-1}, gonadal development was up to level II, and in the treatment of 75 µg g^{-1}, commercial serotonin and ablation, the level was I. The negative control saline solution did not have any effect on gonadal development (Table 2).

Table 2

Observation of gonad maturity level in tiger shrimp (*Penaeus monodon*) treated with banana hump extract.

Treatment with banana hump extract (µg g^{-1} body weight)	*Number of females*	*Gonad maturity level (GML)*				*Percentage (%)*
		I	*II*	*III*	*IV*	
Control	10	-	-	-	-	0
25	10	2	1	1	1	50
50	10	1	1	-	-	20
75	10	1	-	-	-	10
Scom	10	1	-	-	-	10
Ablation	10	1	-	-	-	10

Note: scom - commercial serotonin.

The results of the spermatogenic analysis in male shrimp parents using β-actin as internal control, and LVDmc primer are presented in Figure 7.

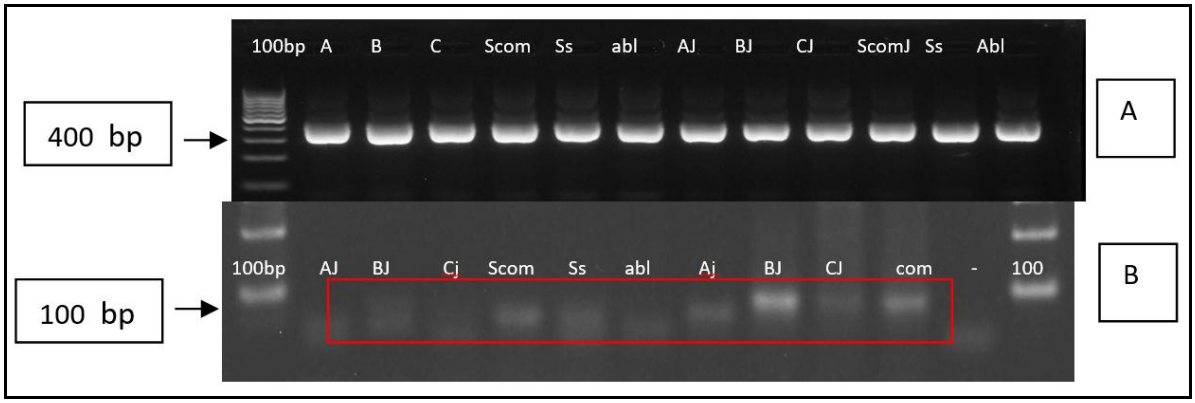

Figure 7. Spermatogenesis analysis on the hemolymph of shrimp (*Penaeus monodon*) males using β-actin as an internal control (A) and primer (B) LVdmc; A - 25 µg g^{-1} body weight; B - 50 µg g^{-1} body weight; C - 75 µg g^{-1} body weight; Scom - commercial serotonin; Ss - saline solution; Abl - ablation.

The results of serotonin isolate application in male shrimp after the third week showed the development of weight, number, and condition of spermatophores (Table 3).

Table 3

Weight and number of spermatophores in prospective tiger shrimp (*Penaeus monodon*) broodstocks after application of three concentrations of serotonin isolates from banana humps compared to commercial serotonin, negative, and positive control

Treatment	*Spermatophore weight (g)*	*Number of spermatozoa*
A - saline solution (negative control)	-	-
B - 25 µg g^{-1} body weight	0.047±2.3	82.4±3.36
C - 50 µg g^{-1} body weight	0.945±1.4	122.6±4.03
D - 75 µg g^{-1} body weight	0.485±1.6	112.7±4.02
E - commercial serotonin	0.020±1.3	50.3±2.23
F - eyestalk ablation (positive control)	0.352±2.7	80.5±2.42

Total hemocytes in shrimp broodstock after application of serotonin isolate from banana hump can be seen in Table 4.

Table 4

Total hemocyte in shrimp brood after application of serotonin isolate from banana humps.

Treatment	*Number of hemocytes (x 10^6) cells mL^{-1}*	
	Female	*Male*
A - saline solution (negative control)	342±2.31	388±2.78
B - 25 µg g^{-1} body weight	685±2.66	720±2.11
C - 50 µg g^{-1} body weight	475±4.31	386±2.34
D - 75 µg g^{-1} body weight	250±1.22	218±4.11
E - commercial serotonin	568±3.12	632±2.43
F - eyestalk ablation (positive control)	867±2.11	786±5.12

Discussion

Isolation of serotonin from banana hump. A plant's serotonin content varies depending on environmental conditions and where it is grown (Erland et al 2015). In bananas, serotonin content is reported to range from 40-150 µg g^{-1} fresh weight

(Ramakrishna et al 2011). The hump of bananas contains serotonin, norepinephrine, tannins, hydroxytryptamine, dopamine, and vitamins A, B, and C. In contrast, the fruit contains flavonoids, glucose, fructose, sucrose, flour, protein, fat, and essential oils, rich in vitamins (A, B, C, and E), minerals (potassium, calcium, phosphorus, Fe), pectin, serotonin, 5-hydroxy tryptamine, dopamine, and noradrenaline (Ramakrishna et al 2011).

UV spectrum measurement. In serotonin, the UV spectrum shows the presence of an aromatic ring (indole ring) which has a π (pi) bond and produces an absorption peak in the UV region. These aromatic rings tend to absorb at wavelengths around 200-300 nm. In addition, the presence of amine groups can also slightly affect the UV absorption spectrum, although the effect is relatively weaker than the aromatic ring (Kiratitanavit et al 2019).

Infrared spectrum measurements. For the measurement of the IR spectrum of serotonin, in addition to FT-IR, some researchers also use IR multiple photon dissociation (IRMPD) spectroscopy. The spectrum was recorded in a Fourier transform ion cyclotron resonance mass spectrometer coupled to an electrospray ionization source and an IR-free electron laser (Lagutschenkov et al 2011).

Survival rate. The results of the application of serotonin isolate from the banana hump, commercial serotonin, saline solution (negative control), and eye stalk ablation (positive control) produced the highest survival in female parents, namely in treatments B and C, followed by treatment A, in contrast to treatments E, D, and F. Male parents had the same pattern (Figure 2). Serotonin isolates from banana humps produce higher survival rates because of serotonin, neurotransmitters, and the content of flavonoids and tannins, which are antimicrobial compounds present in banana hump extract (Pricilia et al 2017). In the negative control of the saline solution, complete mortality was observed. This was likely due to disease. Serotonin has an important role in regulating various body functions, including mood, appetite, sleep, and reproduction (Gonçalves et al 2022).

Molting frequency. Molting is the process of changing the shell in crustaceans and occurs when the size of the organism increases more than the exoskeleton can support (Lemos & Weissman 2021). Thus, to adapt to this situation, shrimp will shed the old exoskeleton and reshape it with the help of calcium (Lemos & Weissman 2021). Molting can be influenced by several factors, including stress or rapid changes in the surrounding environment (Rosmiati et al 2016). Hormonal compounds affected include ecdysterone and serotonin compounds that can control shell turnover in shrimp (Promwikorn et al 2004).

Development of the gonad. The level of gonad maturity of tiger shrimp parents is influenced by several factors, including the weight and condition of the shrimp, the environment, and feed (Okumura 2004). The shrimp broodstock treated with 25 μg g^{-1} BW showed the best response to the banana extract. Serotonin injection for ovarian maturation and spawning of vannamei shrimp has been carried out by Vaca & Alfaro (2000). The results show the best development of ovaries and spawning at a dose of 50 ug g^{-1} BW. Serotonin (5HT) plays a role in the reproductive process in the pelagic *Portunus* crab, especially in oocyte development, which was analyzed through immunohistochemical and tissue expression of receptors using RT-PCR. Serotonin was detected in pairs of neurons and fibers connected to neurons, while in the ovary it was seen in oocytes, indicating the role of serotonin in the reproductive process in crustaceans (Nakeim et al 2020).

The development of Spermatophore. The highest number of spermatophores was in shrimp from treatment C, followed by D, B, and E. The results showed a significant difference when compared to commercial saline solutions and serotonin control

treatments. As a neurotransmitter for induction in shellfish, serotonin can spur the development of spermatophores in male parents (Wang et al 2020).

Total hemocyte count (THC). Shrimp has a non-specific natural resistance to pathogenic organisms in the form of physical, chemical, cellular, and humoral defenses. Genetic and environmental factors influence this natural resilience, so it can be assessed depending on the strain, environment, species, or family. Humoral hemocyte cells are very important as a defense of the body against the attack of pathogenic organisms in shrimp (Reinsel et al 2001). Usually, hemocyte cell counts have a close relationship with the environment. If shrimp live in an unfriendly environment, then hemocyte activity will increase, while if shrimp live under normal conditions, then hemocyte activity remains stable (Rosmiati et al 2022).

Conclusions. The application of banana extract can improve survival and increase the frequency of molting in *P. monodon* broodstock. The optimal dose of banana extract for females was 25 μg g^{-1} BW, while in males, it was 50 μg g^{-1} BW. Banana hump extract containing serotonin can be used to improve the quality of shrimp broodstock.

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Conflict of Interest. The authors declare that there is no conflict of interest.

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