



The Potential of Microencapsulation of Trash Fish-Based Feed Enriched with *Sauropus androgynus* Leaf Extract to Increase Ovary Maturity Rate of Mangrove Crab (*Scylla serrata*)

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Abstract

Mangrove crabs (*Scylla serrata*) are one of the leading fishery commodities in North Kalimantan and have high export value. One of the factors in the development of ovarian maturity in mud crabs is through feeding. This research aimed to assess the nutritional levels and maturity of the ovaries of mud crabs using encapsulated feed enriched with *S. androgynus* leaf. The research design used was a Completely Randomized Design (CRD) with three treatments and three replications. Mangrove crabs are reared for 20 days, feeding 5% of body biomass. The treatments in this study were Control (Trash Fish Feed), A (Trash fish + *S. androgynus* leaf extract), and B (Encapsulation). Microscopic observations showed that the encapsulated feed formed microencapsulated granules. The nutritional content of feed, including protein, fat, carbohydrates, and ash from test samples, showed that treatment B provided optimal nutritional content values compared to other treatments. Observation of ovarian maturity morphology showed TKG IV at 20 days of testing with encapsulated feed. The gonad maturity index (GMI) value of the encapsulated feed gave the highest value and significantly differed from the control and non-encapsulated treatments. The hepatopancreatic index (HSI) value is inversely proportional to the GMI value due to being synthesized into oocytes in the mangrove crab ovaries, thus reducing the HSI value. Therefore, it can be concluded that morphological observations, gonad maturity index, and hepatopancreas index, feed enriched with encapsulated *S. androgynus* leaf extract shows more significant performance in increasing the rate of gonad maturity of mud crabs.

INTRODUCTION

Mangrove crab (*Scylla* sp) is one of the leading fishery commodities in North Kalimantan, with a high export value. However, high market demand needs to be

balanced with the increasing productivity of mud crab cultivation. One factor that influences productivity is gonad maturity. Mangrove crabs take a long time to reach

gonad maturity, so spawning and seed production are slower (Nadia *et al.*, 2022).

Several studies have been carried out to overcome the problem of mangrove crab gonad maturity, such as hormonal induction and feeding food enriched with additive extracts (Awaludin, 2020). One natural ingredient that has the potential to accelerate gonad maturity is *S. androgynus* leaf. It has been proven to expedite gonad maturity in mud crabs. However, feed enriched with *S. androgynus* leaf extract can be damaged if stored for a long time, resulting in reduced nutritional value. This causes the dietary benefits obtained by mud crabs to be not optimal (Jemarus *et al.*, 2023).

Encapsulation technology can be a solution to maintain the nutrition of enriched feed. Encapsulation is the process of coating the core substance with a polymer wall layer so that it becomes micro-sized particles. This technology can protect the active substance from external influences, maintain the stability of the core substance, and extend the shelf life of the feed. The technique used to encapsulate this feed is a gelatin coacervation system (Ulumi *et al.*, 2021). Gelatin coacervation is a process that involves the microscopic encapsulation of the core substance. Acts as a coating that protects the core substance from external influences and controls its slow release.

Based on the background above, an encapsulated feed was made to increase the rate of gonad maturity in mud crabs. This research aims to examine the nutrition of feed enriched with *S. androgynus* leaf extract using encapsulation technology and to explore the effectiveness of encapsulation of feed enriched with *S. androgynus* leaf extract on the rate of gonad maturity of mud crabs, including the rate of gonad development, gonad maturity index (GMI), and hepatopancreas index (HSI).

METHODOLOGY

Ethical Approval

All stages of research procedures were carried out according to standards, both in

laboratory and field testing. Analysis of the test biota was carried out by physical observation every day by providing food according to the test treatment.

Place and Time

The research was conducted from April to July 2024 at the Tarakan Pamusian Crab Breeding Pond, Fish Nutrition and Feed Laboratory, Water Quality Laboratory, and Life Sciences Resources Laboratory (LSIH), University of Borneo Tarakan.

Research Materials

The raw materials in this research are *S. androgynus* leaf, trash fish, gelatin, egg white, distilled water, and ethanol p.a (Merck). The materials used to analyze the nutritional content of encapsulated feed are BSA (Bovine Serum Albumin) solution (Sigma Aldrich), anhydrous glucose p.a (merck), biuret reagent (Sigma Aldrich), arsenomolybdate (Sigma Aldrich), NaOH (Merck), HCl (Merck), Nelson A (Sigma Aldrich) reagent, Nelson B (Sigma Aldrich) reagent, and Whatmann filter paper no.41. The tools used in this research are silicone moulds, dehydrators (Wirastar FDH-6; Indonesia), mixers (Maspion, MT-1150; Indonesia), ovens (Mettler; German), blenders (Miyako BL-152; Indonesia), UV-Vis spectrophotometers (Shimadzu; Japan), desiccators (Schoot Duran; German) and hot plates (IKA C-Mag HS 7; German).

Research Design

This research used a Completely Randomized Design (CRD) with three treatments and three replications. Mangrove crabs are reared for 20 days, feeding 5% of body biomass. The research design consists of Control: Trash fish feed, Treatment A (Trash fish meal + *S. androgynus* leaf extract), and Treatment B (Encapsulated feed).

Work Procedure

Microencapsulated Feed Nutrition

Each treatment was tested for feed nutrition. First, test the protein feed's

nutrition by determining the wavelength of the standard BSA solution and protein content by spectrophotometry using a biuret reagent. Test fat nutrition using the Soxhlet method. The nutritional test for carbohydrate feed is first by determining the wavelength of a standard glucose solution and carbohydrate content by spectrophotometry using Nelson A and B reagents. Meanwhile, the test for water content and ash content uses the feed proximate test.

Gonad Maturation Rate (Ovarian Maturation Stage, Gonad Maturity Index, and Hepatosomatic Index

Macroscopic morphological observations of the ovaries were carried out. A surgical procedure to observe the level of gonad maturity, following the procedure of Awaludin (2020), was used to observe the gonads.

Observation of the Gonad Maturity Index (GMI), namely the weight of the gonad obtained divided by the total body weight and multiplied by 100%.

$$GMI = \frac{\text{Gonad Weight (g)}}{\text{Body Weight (g)}} \times 100\%$$

Observation of the Hepatosomatic Index (HSI), namely the weight of the hepatopancreas obtained divided by the total body weight multiplied by 100%.

$$HSI = \frac{\text{Hepatopancreas Weight (g)}}{\text{Body Weight (g)}} \times 100\%$$

Sample Preparation

Each *S. androgynus* leaf and trash fish are washed clean and dried in an open room and oven at 60°C until completely dry. Once dry, each is ground using a blender until it becomes powder or flour.

Extraction of *S. androgynus* Leaf

1 kg of *S. androgynus* leaf powder was macerated with 3 L of 70% ethanol solvent for 3 x 24 hours. The macerated extract is then filtered and the filtrate is taken. The filtrate solution is evaporated until it becomes *S. androgynus* leaf extract paste,

then dried in a desiccator (Kartina *et al.*, 2024).

Control Feed

Add one egg white, which has been mixed as an adhesive, to the trash fish flour. After mixing the feed, it is molded in a silicone mold and oven at 60°C until dry (Control Treatment).

Trash Fish Meal Enriched with *S. androgynus* Leaf Extract

S. androgynus leaf extract is added to the trash fish flour in a ratio of 10:1, after which one egg white mixed as an adhesive is added. After mixing the feed, it is molded in a silicone mold and oven at 60°C until dry (Treatment A).

Encapsulated Feed

Trash fish flour is mixed with *S. androgynus* leaf extract and gelatin in a ratio of 1:0.1:5. The ingredients are combined with additional water and mixed until homogeneous for 15 minutes (emulsion occurs). When the emulsion is formed, it is heated via a hot plate until it reaches a temperature of 80-90°C. The process of increasing feed can use a portable stove for heating. Then the feed is molded and placed in the oven at a temperature of 60°C. After drying, the feed is cooled and ready to be tested (Treatment B).

Data Analysis

The gonad maturity index and hepatopancreas index data obtained in this study will be processed using ANOVA statistical data with a confidence level of 95% using SPSS 25. Duncan's further test will be carried out if they are significantly different. Guidelines for decision-making are as follows: if the significance value is > 0.05, then there is no significant difference in the rate of gonad maturity between feed treatments; if the significance value is < 0.05, then there is a significant difference in the rate of gonad maturity between feed treatments. If it is significantly different, the

Duncan test will be continued to get the best treatment.

RESULTS AND DISCUSSIONS

Feed Test

The feed made as test feed consisted of control feed, namely trash fish meal,

treatment A feed, namely trash fish meal and *S. androgynus* leaf extract without encapsulation, and treatment B feed, namely trash fish meal and *S. androgynus* leaf extract with encapsulation. The following is a picture of the feed and morphology resulting from microscope observations with 10x magnification.

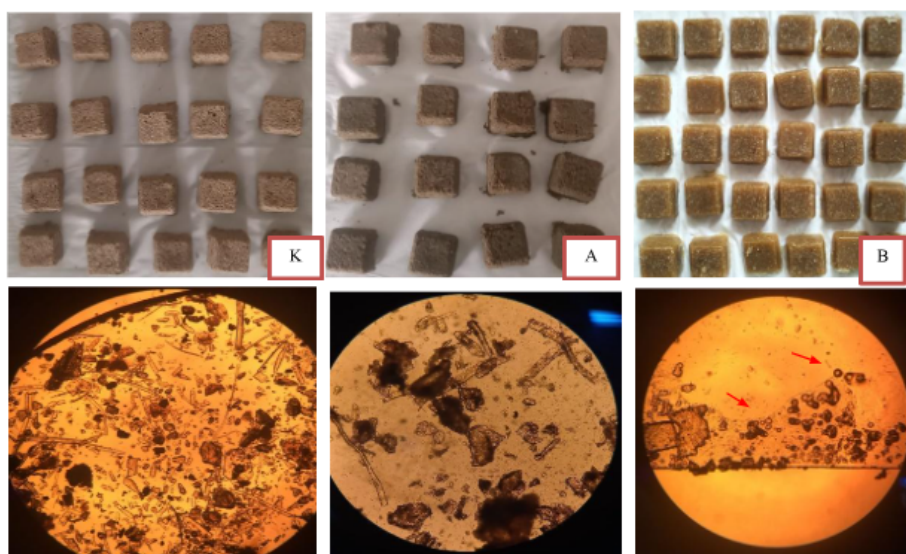


Figure 1. Feed test.

Description: (K) Control, (A) Treatment A, (B) Treatment B.

Based on the results of the microscope observations, it can be seen that the treatment B feed forms microencapsulation. This is different from other forms of feed (control and treatment A). In treatment B, gelatin acts as an encapsulation agent, coating the mixture of trash fish meal and *S. androgynus* leaf extract to form a small round layer (Aminah and Hersoelistyurini, 2021). Encapsulated feed that is successfully formed will look like a small capsule (red arrow) and is composed of a layer of gelatin that encloses the encapsulation core, namely the active ingredient that you want to protect, in this case, trash fish meal and *S. androgynus* leaf extract (Umayah, 2022).

Feed Nutrition

One way to obtain optimal results in accelerating crab gonad maturity is by improving the nutritional quality of parent feed. The test feeds included control feed from trash fish, trash fish feed enriched with *S. androgynus* leaf extract, and trash fish feed enriched with encapsulated *S. androgynus* leaf extract. The nutrients measured include protein, fat, carbohydrates, and ash content. The following are the nutritional levels of each test feed.

Table 1. Feed nutrition.

| Treatments | Feed Nutrition | | | |
|-------------|----------------|-------|--------------|-----|
| | Protein | Lipid | Carbohydrate | Ash |
| Control | 37.91% | 0.4% | 10.16% | 19% |
| Treatment A | 53.66% | 4.4% | 17.5% | 17% |
| Treatment B | 59.16% | 2.6% | 22.16% | 3% |

Based on the table data above, the highest protein and carbohydrate nutritional content values were in treatment B (encapsulated feed). The highest fat nutrition was in treatment A, while the control treatment had the highest ash content. Optimal nutrition will influence the rate of gonad maturity. Protein is the primary nutrient needed in the reproductive process. This is because the essential ingredients in the formation of egg cells and sperm cells come from the metabolic results of the feed given, especially to female fish; this maturation process is known as the vitellogenesis process (Parente *et al.*, 2024). The essential ingredients in the gonad maturation process consist of carbohydrates, fat, and protein.

Aryani *et al.* (2014) reported that giving different amounts of protein feed affected the time to achieve gonad maturity in baung fish, with a protein content of 37% resulting in the fastest gonad maturity, namely 26 days. The protein content is obtained from trash fish, but apart from protein, fat also plays a role in increasing gonad maturity. The function of fat in gonad maturation is for cell structure and integrity of biomembranes. In the control feed, which is often used by farmers, the fat content is very low; therefore, it needs to be enriched with additional ingredients.

One of the fatty acids used for gonad maturity is cholesterol. Cholesterol is needed to fulfill several endocrine

functions, namely as a precursor of steroid hormones, for gonadogenesis, ovarian maturation, and larval development (Wouters *et al.*, 2001). Cholesterol is contained in *S. androgynus* leaves in the class of steroid compounds. Based on the nutritional results, it can be seen that feed enriched with *S. androgynus* leaf extract increases the fat content in the feed. Awaluddin *et al.* (2020) stated that steroids are needed for cholesterol endocrine function, namely as a precursor to steroid hormones for the process of gonadogenesis, ovarian maturation, and larval development (Awaluddin *et al.*, 2020). Encapsulated feed, in addition to a gelating coating, maintains the nutrition of the feed inside. Adding gelatin, which is high in protein and is a coating, adds protein nutrition to feed (Ulumi *et al.*, 2021). Therefore, feed nutrition is balanced to increase gonad maturity.

Mud crab gonad maturity rate Ovarian maturation stage

Observations on the rate of gonad maturity of mud crabs were carried out for 20 days, with sampling every 10 days. During the test, test feed was given according to the treatment, amounting to 5% of mangrove crab biomass. The following are the results of observing the morphology of mangrove crabs.

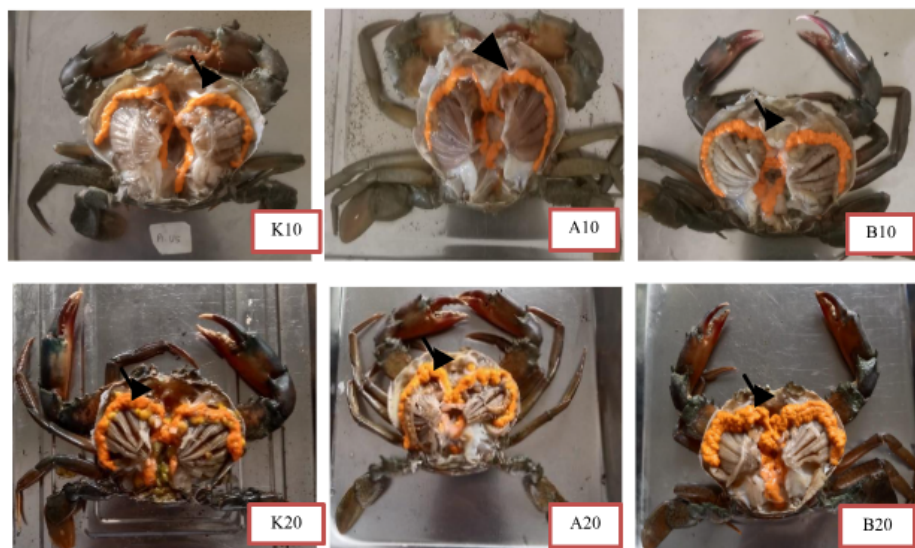


Figure 2. Ovarian morphology.

Description: (K10) Day 10 Control, (A10) Day 10 Treatment A, (B10) Day 10 Treatment B, (K20) Day 20 Control, (A20) Day 20 Treatment A, (B20) Day 20 Treatment B.

Determining the level of gonad maturity morphologically, as is done in fish, includes shape, length and weight, color, and visible development of gonad contents (Effendie, 1997). The maturity level of mud crab ovaries can be classified into five stages based on external characteristics and the presence of oocytes (Islam *et al.*, 2010). Based on visual morphology, the gonad maturity of mud crabs can be seen in Treatment B, indicating a higher maturity rate than other treatments. On the 10th day of sampling, the K10 sample showed early TKG II, and the A10 sample was at the final TKG II level, which is characterized by thin gonad morphology and yellow color. Meanwhile, sample B10 has entered the initial TKG III gonad maturity level. On the 20th day, samples K10 and B10 entered the initial TKG III gonad maturity level. It is shown that the color of the gonads starts to become thicker with the number of gonads starting to cover the pancreas.

Meanwhile, B20 experiences development at the secondary vitellogenic stage, namely TKG IV. It can be seen from the shape of the ovaries getting bigger, the

egg cells are dark yellow and fill almost half of the abdominal cavity. This is in line with research (Awaludin, 2020), which shows that *S. androgynus* leaf extract can increase the gonad maturity of mud crabs, as seen on the 10th day of sampling. However, on the 20th day of sampling, the encapsulated feed performed better, while the feed enriched with *S. androgynus* leaf extract without encapsulation tended to be the same as the control treatment. This is because the longer shelf life of feed can reduce its nutritional performance. This differs from encapsulated feed because nutrients can be protected from external influences due to the encapsulation coating layer so that the feed lasts for an extended period (Putra, 2022; Wati *et al.*, 2022).

Gonad Maturity Index (GMI)

GMI calculations were carried out for 20 days, with sampling every 10 days. The GMI calculation is obtained from the percentage ratio of gonad weight to crab body weight. The following are the GMI results from the test feed treatment.

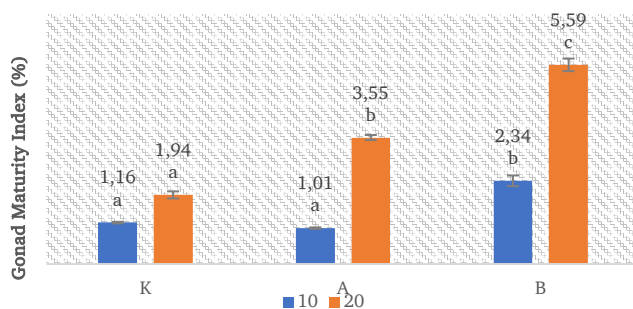


Figure 2. Gonad maturity index percentage. The results presented are based on data normalization. Different superscripts on the same rearing day show significant differences at the 95% confidence level ($P < 0.05$).

Description: (K10) Day 10 Control, (A10) Day 10 Treatment A, (B10) Day 10 Treatment B, (K20) Day 20 Control, (A20) Day 20 Treatment A, (B20) Day 20 Treatment B.

At sampling on both days 10 and 20, it was seen that treatment B showed a more significant increase in GMI compared to control treatment and A. Treatment B contained feed raw materials that were richer in nutrients that were important for gonad development, such as protein, carbohydrates, and minerals. This is supported by the sterol compound content of *S.androgynus* leaf extract added to the feed, which causes the acceleration of gonad maturity in mud crab parents (Awaludin, 2020). In addition, encapsulated feed protects the feed's nutrition, so even if it is stored for a long time, the nutritional content needed for gonad maturity is maintained (Ulumi *et al.*, 2021). Therefore, the GMI value of encapsulated feed shows better performance. Based on statistical analysis, both sampling days 10 and 20 showed a sig value < 0.05 , indicating a significant difference in treatment B (encapsulation)

compared to other treatments.

Hepatosomatic Index (HSI)

The hepatosomatic index (HSI) describes the hepatopancreas in crabs. The HSI value was observed to observe the vitellogenesis process in mangrove crabs for oocyte development. Based on the research results, the HSI value can be seen in Figure 3. The HSI value shows that the more the ovarian cells develop, the smaller the HSI value becomes. This means that the ovaries have used vitellogenin synthesis for oocyte development (Hanif and Herlina, 2021).

On days 10 to 20, the HSI value is inversely proportional to the GMI value. This is supported by the statement (Farizah *et al.*, 2017) that the transfer of vitelline from the hepatopancreas to the ovaries increases the GMI value, which decreases the HSI value in mud crabs due to the vitellogenesis process.

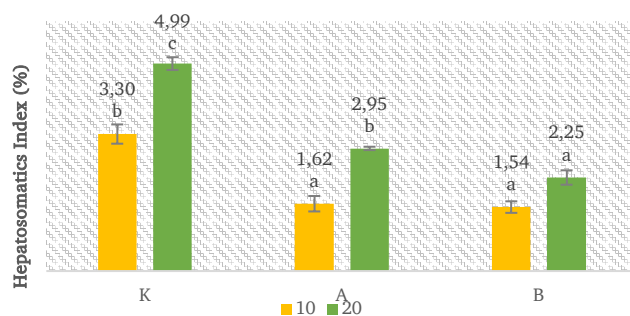


Figure 3. Hepatosomatic Index Percentage. The results presented are based on data normalization. Different superscripts on the same rearing day show significant differences at the 95% confidence level ($P < 0.05$).

Description: (K10) Day 10 Control, (A10) Day 10 Treatment A, (B10) Day 10 Treatment B, (K20) Day 20 Control, (A20) Day 20 Treatment A, (B20) Day 20 Treatment B.

Based on statistical analysis, a sig value < 0.05 showed a significant difference between the test treatments, but on day 10, samples from treatment A and treatment B were not significantly different. This is because both are enriched with *S. androgynus* leaf extract. Still, they are significantly different on the 20th day, because the performance of unencapsulated feed begins to decline due to shelf life, and is inversely proportional to the encapsulated samples which can maintain feed nutrition.

CONCLUSION

Encapsulated feed was successfully formed through gelatin coacervation. Based on the research results observing morphology, gonad maturity index, and hepatopancreas index, feed enriched with encapsulated *S. androgynus* leaf extract showed more significant performance in increasing the gonad maturity rate of mud crabs.

CONFLICT OF INTEREST

There is no conflict of interest among all authors upon writing and publishing the manuscript.

AUTHOR CONTRIBUTION

MSL as a research coordinator and drafted the publication article. SF carried out sample preparation and processed and calculated data. IAO researched *S. androgynus* leaf extract extraction and

microscope observations of feed. NAP writes research notes in the logbook and carries out feed nutrition tests. E carries out maintenance of mud crabs and tests the gonad maturity of mud crabs.

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