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Effect of Several Natural Feeds on Increasing the Maturity of the Ovaries of the Broodstock of Mub Crabs (*Scylla Serrata*)

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ABSTRACT

This research aimed to show the natural feed effect on improved matured ovary broodstock mud crab (*Scylla serrata*). The research was conducted on Tarakan Island, North Kalimantan, Indonesia. The mud crabs used consisted of heads, weighing 300-400g, with the ovary at maturity level II stage. The feed used in this study consisted of fresh ingredients, including anadara shellfish (*Anadara granulosa*), seaworms (*Polychaeta*), and beef liver. These ingredients are available in large quantities and have not been used for mud crab broodstock. The parameters observed include ovarian maturity, fecundity, and water quality. This study used a complete randomized design (RAL) with three treatments and six repeats. An analysis of data on the acceleration of the ovary maturity rate was carried out using ANOVA. The treatment differentiation of fresh feed resulted in a significant (P<0.05) time maturity ovarium. *A. Granulosa* feeding is considered extremely good for triggering the acceleration of berries in mud crab broodstock ovary. Fresh feed treatment provides different fecundity values for mud crab broodstock, but based on ANOVA analysis, it shows no different results. Feeding fresh *Anadara granulosa* and beef liver clams accelerates the development of mud crab ovary compared to other diets. These ingredients have higher levels of proteins and a total lipid, making them beneficial for the formulated diet of mud crab broodstock.

INTRODUCTION

The primary species of focus for aquaculture in traditional ponds in North Kalimantan, Indonesia is the mud crab (*Scylla serrata*). Mud crabs have commercial value, especially mature female crabs with mature ovaries. They can be found in mangrove ecosystems, estuaries, and tidal zones throughout the Indo-Pacific region. Mud crabs are commodities in temperate and tropical waters **(Bian *et al*., 2022; Liu *et al*., 2022)**.

Ovarian quality is influenced by diet or nutrients that are absorbed and accumulated into biochemical substances in the ovaries and hepatopancreas which can affect the reproductive stages **(Wu *et al*., 2020; Farhadi *et al*., 2021; Farhadi *et al*., 2022)**. Mud

crab cultivation has challenges, such as low brood quality, slow development of ovary crabs, and poor egg quality. Mud crab broods still produce low fecundity and low larval survival. This is thought to be because mud crab broodstock is only given natural feed as trash fish, which is insufficient to meet specific nutritional needs. In this area, there are plenty of fresh ingredients that are readily available and easy to find but have never been utilized, such as Anadara shellfish (*Anadara Granulosa*), sea worms (*Polychaeta*), and beef liver.

Mud crab cultivation requires quality brood. The broodstock nutrition should first be known before further breeding or restocking programs. Good feed can support the quality of mud crab broodstock. Based on some studies, giving fresh feed to broodstock mud crabs can increase the fertility of the mother **(Azra & Ikhwanuddin, 2016)**.

Increasing the production of good mud crabs can be through feed whose raw materials are available and controlled **(Nguyen *et al.*, 2022)**. Good feeding of mud crab broodstock can improve the quality of egg laying and adaptability to the environment. Moreover, it will help increase the crab's immunity **(Yue *et al.*, 2023)**. Lipid content in food functions as an essential nutrient and the main energy source for embryo development and reproductive performance of female crabs. Lack of lipid reserves during spawning can damage ovarian maturation and larval survival in *Scylla* sp. **(Lin *et al.*, 2021; Prakash *et al.*, 2021)**.

Appropriate maturation diet is crucial for promoting the reproductive performance of the mud crab broodstock, if the mangrove crab broodstock feed lacks nutrition, it will disrupt the development of the ovaries and the quality of crab eggs. Mud crab broodstock needs several essential nutrients, such as fatty acids, phospholipids, and sterols, which are indispensable for reproduction, ovarian development, spawning, embryogenesis, and larval development **(Ren *et al.*, 2022)**. Anadara shellfish (*Anadara granosa*), seaworms (*Polychaeta*), and beef liver are available in large quantities but have never been used for mud crab broodstock. This is thought to be due to the unknown nutritional value of these feed, which makes this research important. The present study aimed to determine the effect of natural feed in this area on improved matured ovary broodstock mud crab (*Scylla serrata*).

MATERIALS AND METHODS

1. Materials

The females of *S. serrata* in various maturation stages were obtained from traditional ponds and mangroves in Tarakan Island, North Kalimantan, Indonesia. This research was conducted on Tarakan Island. The mud crabs used were 15 heads, weighing 300-400g, with an ovary maturity stage II. The feed used in this study was fresh feed consisting of Anadara shellfish (*Anadara granosa*), seaworms (*Polychaeta*), and beef liver.

2. Methods

The selected broodstocks were reared in plastic containers having a volume of 100L with a density of 1 ind./container. Sand was added to the bottom of the container to suit natural conditions. 50L of water was used, and the quality was maintained by using a closed water circulation system. Mud crabs were fed twice a day (morning and evening), with the feed amounting to 5-8% of the crab's total weight. The parameters observed include ovarian maturity, fecundity, gonado-somatic index (GSI), hepatosomatic index, HSI and water quality. This study used a complete randomized design with three treatments, Anadara shellfish (*Anadara granosa*), seaworms (*Polychaeta*), and beef liver, and six repeats. The analysis of data on the acceleration of the ovary maturity rate was carried out with a one-way ANOVA.

RESULTS

Feed is one of the essential factors in the development of the livestock business. The success of the livestock business depends on feed management. Feed given to livestock must contain necessary nutrients needed by the livestock body while maintaining a balanced composition. The fresh feed used, such as blood mussels (*Anadara granosa*), beef liver, and sea worms (*Polychaeta*), and their nutritional content can be seen in Table (1). Moreover, the development of the maturity level of mud crab ovary with different natural fresh feed is exhibited in Table (2). The results in Table (3) show that GSI values in *A. Granosa* and beef liver feeds had the highest average compared to Polychaeta feed treatments. This is thought to be because the proximate protein and fat content of mussels (*Anadara granosa*), and beef liver, is higher than that of sea worms (*Polychaeta*). Based on Table (4), the HSI value decreased due to the vitellogenesis process in mud crabs. The analysis of variance of the fresh feed in the female *S. Serrata* indicates a natural effect in the acceleration of mature ovaries. The treatment differentiation of fresh feed resulted from a significant ($P<0.05$) time maturity ovarium. *A. Granulosa* feeding is considered very good for triggering the acceleration of berries in mud crab broodstock ovary. This means that treatment differentiation of fresh feed can affect the acceleration of mature ovarium in the process of vitellogenesis.

Based on Table (5), fresh feed treatment provides different fecundity values for mud crab broodstock. However, no significant results were shown using the ANOVA analysis ($P>0.05$). Based on other studies, the use of fresh natural feed can increase the fecundity of crabs so that it can indirectly increase the number of eggs **(Azra & Ikhwanuddin, 2016)**. The highest fecundity in the treatment was in the use of *A. granulosa* feed, which was around 1.50×10^6 eggs or 1.500.000 (Table 3). This result is different from research by **Davis *et al.* (2004)**, which states that the broodstock mud crab in Africa has around 1,000,000 eggs.

The parameters of water quality measurements during the rise are presented in Table (4). The results of measuring water quality in Tarakan Island locations show values primarily by survival life and culture of mud crabs.

Table 1. The nutritional content of fresh feeds

Fresh Feed	Carbohydrate	Protein	Lipid	Water	Ash
Anadara granosa	16,97%	45,92%	7,11%	11,25 %	3,78 %
Beef liver	5,27%	38,93%	25,8%	12,16 %	3,24 %
Polychaeta	35,71%	24,33%	2,96%	11,78 %	3,52 %

Table 2. Data (mean ± SD) the ovarium mature of female mud crab

Treatment	Mature of ovarium (%)	Stage II-III (days)	Stage III-Berried (days)	SR (%)
Anadara	73,3 (n=15)	20±3.83[a]	13±26.00 [a]	86.7 [a]
Beef liver	60,0 (n=15)	25±2.2.0[ab]	18±14.27 [a]	80,0 [a]
Polychaeta	40,0 (n=15)	28±14.00[b]	26±42.31 [b]	66,7 [b]

Means in the same column with the same superscripts under source of treatments and treatment are significantly different (P<0.05).

Table 3. Gonad somatic index

Treatment	GSI day to- (%)			
	7	**14**	**21**	**28**
Polychaeta	5,81±2,52	8,34±1,53	11,95±2,08	13,50±2,65 [b]
Anadara granosa	7,51±2,65	10,33±1.00	14,50±2,08	18,3±4,16 [a]
Beef liver	8,43±1,53	10,60±2,08	13,71±1,00	17,6±8,14 [a]

Means in the same column with the same superscripts under source of treatments and treatment are significantly different (P<0.05).

Table 4. Hepatopancreas somatic index

Treatment	HSI day to- (%)			
	7	**14**	**21**	**28**
Polychaeta	4,71±0,58	4,00±1,00	3,47±1,53	3,10±1,08 [b]
Anadara granosa	4,24±0,58	3,62±0.58	3,11±1,53	2,1±1,75 [a]
Beef liver	4,91±1,15	3,41±1,00	3,05±1,15	2,0±1,53 [a]

Means in the same column with the same superscripts under source of treatments and treatment are significantly different (P<0.05).

Table 5. Rate data (mean ± SD) fecundity of mud crabs broodstock *S. serrata*

Treatment	Weight (g)	Fecundity (egg)
A. granosa	338.7±23.83[a]	1.50 ×10[6a]
Beef liver	347.2±13.27[a]	1.49 ×10[6a]
Polychaeta	344.1±20.00[a]	1.41 ×10[6a]

Means that the same column with the same superscripts under the source of treatments and all treatments are‘t significantly (P>0.05).

Table 4. Results of measurement of water quality parameters.

Parameter	Rate
Temperature ^{o}C	26 ^{O}C – 30^{O}C
pH	7,3 -7,49
DO	5,5 – 6,51
Amoniac	0,01 – 1,0
Salinity	25 ppt – 30 ppt

DISCUSSION

Anadara granosa is known as the blood cockle due to its red color, which results from the presence of red hemoglobin fluid in its soft tissue. It can be found in the Indo-Pacific region from the east coast to the north of South Africa and east to Southeast Asia, Australia, Polynesia, and as far north as Japan. *Anadara granosa* lives in tidal areas with water depths of around 1-2 meters and often hides in sand. Sea worms (Polychaeta) are a group of marine worms/annelids. These worms are segmented, about 5-10cm long, their color is bright, and have feathers called chaetae. The worms commonly found in this area are found in the sand near the mangrove trees. Beef liver comes from beef cattle and is easy to obtain in the market, and the price is also cheap.

Feed containing good nutrition can directly affect the maturation of the ovaries of the parent mud crab and limited food nutrition can inhibit the maturation of the crab ovaries **(Alava *et al*, 2007)**. The ovarian maturation period for mud crabs (*Scylla* spp.) is a process that takes around 55-60 days, depending on environmental, hormonal, and genetic conditions and enzyme activity **(Hidir *et al*., 2021)**. The process of ovary maturation is the formation of vitellin, the primary molecule of crustacean egg yolk protein, which plays an essential role in embryo development. In *Scylla serrata*, the vitellogenesis process occurs in the hepatopancreas and ovaries **(Subramoniam, 2011)**.

A nutritional test of fresh feed was conducted to identify the option that can accelerate the development of mud crab ovary. Mud crab broodstock has a high dietary requirement for lipids, fatty acids, and protein used during maturation and reproduction.

Based on research, mud crab broodstock needs feed containing 32-40% protein and 6-12% lipids for their growth **(Azra & Ikhwanuddin, 2016)**. Lipid content is needed in the maturation process of mud crab broodstock ovary **(Aaqillah-Amr *et al*., 2021)**.

Table (2) shows the reproductive performance quality of mud crab broodstock, genus *Scylla* fed with various natural diets. In general, the natural feed affected mud crab broodstock performances. It is suggested that the natural diets can improve the broodstocks fecundity which indirectly increases the number of eggs produced from a single broodstock.

The development of the crab ovary is a process of vitellogenesis in which vitellogenin is prepared in the yolk. The process of synthesis of yolk and egg white components occurs in the liver and oviduct, which is indirectly affected by nutrients.

Vitellogenin is an egg yolk protein needed as a nutrient for larval development. Vitellogenin content consists of lipo-glycol-phosphoprotein expressed in the liver, and the nutritional composition of eggs consists of 30%-32.5% lipids, including various sterolipids, acyl fatty esters, sphingolipids, isopentenolipids, glycolipids, and glycerophospholipids **(He *et al*., 2023)**.

Protein in feed may lead to essential amino acid deficiency, which can reduce the efficiency of using protein for egg development. The protein content in a high feed can accelerate the ovary maturation process because the amino acid content is more complete than the lower protein content of a feed. Based on the research results on the development of mature ovary mud crab, the fastest is found in a high protein content feed, namely fresh feed *A. granulosa* and beef liver. The maturation process of eggs in *S. serrata* increases with clover's high protein content (Table 2). This is thought to be because the protein provides energy for the development of crab eggs. The feed energy consumed by organisms is used for growth energy that enters through the amount of feed consumed sourced from proteins, fats, and carbohydrates, as the primary energy source. This result agrees with the finding of **Aaqillah-Amr *et al*. (2021)** that mud crab broodstock given commercial fresh food twice a day can accelerate ovarian maturation.

The results of studies have shown that the lipid content in the preparation of egg yolks plays a significant role in shaping the quality of egg yolks. Egg yolks mainly comprise lipids: triglycerides (TG), phospholipids, and cholesterol. Egg yolks are an essential source of lipids because they are energy providers and have other physiological and biochemical functions. The study by **Millamena and Quinitio (2000)** found that lack of essential dietary fatty acids in natural diets can reduce the reproductive performance of broodstock. Phospholipids in egg yolks can control biological activities such as antioxidants, antibacterial, and anti-inflammatory activities and regulate lipid metabolism. Cholesterol can be a precursor to bioactive substances such as vitamin D, hormone sterols, and bile acids **(Di Bernardo *et al*., 2021)**.

Fecundity in crabs depends on several factors, including population levels, such as a low proportion of ovarian-mature females resulting in low meeting rates during mating, due to receptive females, and due to limited sperm so that the male population cannot fertilize all eggs. Fecundity can also be affected due to egg loss during long periods of embryogenesis **(Di Salvatore *et al*., 2019)**. This study demonstrates the effects of fresh feed on the fecundity of mud crab broodstock. All three treatments showed no real difference in influencing the amount of fecundity of eggs. This proves that the three fresh feeds still support the fecundity of crab eggs.

The development of crab embryos is always influenced by the availability of nutrients obtained from food. A lack of nutrition in the feed of the mud crab mother will decrease both egg fertility growth and the fertilization process. Nutrients contained in feed such as amino acids, vitamins and essential fatty acids affect embryo development, especially egg morphology, hatching rate and vitellogenin synthesis.

Lack of nutrition during the gonad maturation process can have a negative impact on gonad development. Micronutrient deficiencies cause defects in neural tube closure, bone mineralization, and immune system development **(Wilson *et al*., 2018)**.

CONCLUSION

A sufficient amount of nutrients such as protein, lipids, and carbohydrates should be provided in the broodstock diet to permit the successful development of the mud crabs' ovaries and the quality of eggs produced.

Feeding fresh *Anadara granulosa* and beef liver clams accelerates the development of mud crab ovary compared to other diets, as these ingredients contain higher levels of protein and total lipids, making them highly suitable in the formulated diet of mud crab broodstock.

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