EFFECT OF CALCIUM SUPPLEMENTATION ON GIANT FRESHWATER PRAWN (*Macrobrachium rosenbergii*) MOULTING AND EGG QUALITY

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ABSTRACT

A breeding program for giant freshwater prawn has been developed in Indonesia to supply good quality prawn seed to industry. To achieve the goal of the program, optimum conditions of both environment and nutrition must be provided. Calcium is the main compound of the prawn carapace, influencing moulting processes, especially during the premating moult. The effect of calcium supplementation in the prawn feed on moulting process and egg quality of giant freshwater prawn, *Macrobrachium rosenbergii,* was investigated. Experimental units consisted of 300 L plastic tanks stocked with 4 adult prawns comprising 3 female and 1 male. A standard prawn feed containing 30.39% crude protein; 0.82% Ca and 0.47% P was used as the experimental diet. A complete randomized design was‘applied in the study with 5 dosage levels of dicalcium phosphate i.e. 0%, 2%, 4%, 6%, and 8% with 4 replicates. Prawns were reared over 45 days. There were significant differences ($P<0.05$) in premoult, moult, and egg hatching rate, while no significant differences in intermoult or fecundity were observed ($P>0.05$). Egg hatching rate increased liniearly from 26.5%±9.9% to 50.8%±10.3% as calcium dosage increased from 0% to 8%. The number of eggs per spawn was not significantly different ($P>0.05$), ranging from 40,096 to 46,131 for females weighing 30.19 to 32.94 g. The results of this study suggest that giant freshwater prawns require dietary calcium supplementation to support moulting process and egg quality when reared in soft water.

KEYWORDS: calcium, egg quality, giant freshwater prawn, moulting, spawning

INTRODUCTION

The giant freshwater prawn, *Macrobrachium rosenbergii*, is native to the Indo-West Pacific region (Holthuis, 2000 *in* New & Valenti, 2000; Wowor & Ng, 2007). In Indonesia, there is a strong opportunity to develop farming of the prawn, driven by high price and good markets. Development of prawn farming system must be supported by continuous supply of good quality seed. In Indonesian prawn hatcheries there is evidence of decreased genetic quality of stock indicated by early maturation of females and small size at harvest, which in turn effect productivity and price. To solve the problems, breeding programs for prawns have been established to produce faster growth and disease resistance of seed prawn, and to address high mortality of adult prawns and low numbers of gravid females (Ali, 2006; Khasani *et al*., 2010). The main mortality factors are molting and cannibalism.

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Prawns periodically shed their old exoskeleton, resulting in somatic tissue growth, a process known as moulting or ecdysis (Greenaway, 1985; Ismail & New, 2000). During the moulting process the old cuticle is divested and replaced by a new, initially weaker, cuticle. The period between moults, that is the intermoult period is dependent on prawn size (Passano, 1960; Wickins, 1976) and water hardness (Adikari *et al.*, 2007). The process is a critical event in the life cycle of all crustaceans, and successful moulting is necessary for normal growth and survival of the prawn (Feiber & Lutz, 1982 *in* Sukadi, 1999). During moulting, when the cuticle of the prawn is soft, risk of mortality is high due to both cannibalism and weakened condition of the prawn (Saravanan *et al.*, 2008). The risk may increase in condition of poor water quality. During the maturation processes, the adult female prawn usually moults before mating (Ismail & New, 2000). Thus, moult realited mortality can be a factor in the management of broodstock populations.

The primary problems in prawn selective breeding progams in Indonesia are high mortality rate and low numbers of gravid females during mating in indoor hatcheries with a half-sib matting system (Ali, 2006). It is thought that these problems are atributable to poor suitability of mating tanks and calcium deficiency in both the tank water and prawn diet. During the moulting process calcium availability is very important because the compound is a vital component in creating a new and hard carapace (Passano, 1960; Fieber & Lutz, 1982). Calcium is very important to freshwater prawns in keeping the exoskeleton strong. When calcium is too low prawns will take a longer time after a molt for the exoskeleton to harden, thus prolonging the period in which they are vulnerable to mechenical damage and predators. Prawns exposed to low calcium “soft” water are also more susceptible to other poor water quality conditions (Adhikari *et al.*, 2007).

Calcium levels in freshwater are usually much lower than those in seawater. The range in calcium concentration of typical ground water is between 1 and 120 mg/L (Robertson, 1941 *in* Sukadi, 1999). To counter low calcium level in the water, calcium can be supplemented through the prawn diet. Most commercial prawn feed contain low concentrations of calcium and are not likely to be suffecient to support the calcium requirement of adult prawns. Hakim (2009); Davis *et al.* (1993) reported that calcium supplementation in the feed is an effective method to supply the nutrient for prawn growth.

Based on the above facts, addition of calcium in the prawn feed was suggested to have a potential benefit in reducing duration of the moulting process and increase reproduction performance of the prawn. The following experiment was designed to address recent problems in the prawn mating process in Indonesian hatcheries.

MATERIAL AND METHODS

The present investigation was conducted at the Research Institute for Freshwater Fish Breeding, Subang District, West Java Indonesia. The study was conducted using 20 plastic tanks equipped with water exchange system. The size of each tank was 100 cm x 60 cm x 50 cm and they were filled with 300 L of ground water. Females at the same ovary stage and blue claw males were chosen for this experiment. Each female prawn was tagged with a small numeric tag to monitor each individually, and acclimated for 3 days in the tanks. The mean body weight of prawns used was 30 to 40 g for females and 50 to70 g for males, and they were stocked with a sex ratio 3 females to 1 male. This ratio was maintained throughout the experiment by replacing dead prawns with replacements taken from a prawn reserve tank. Two plastic boxs were placed in each tank to serve as a shelter for protection of premating females from predation. Continuous aeration was provided, and cleaning of uneaten food and wastes was performed daily. A 10-20% of water exchange was made daily to maintain good water quality.

A complete randomised design was applied in the study with 5 levels of dosages of calcium i.e. 0%, 2%, 4%, 6%, and 8% with 4 replicates. The composition of the prawn feed is presented in Table 1. This basal diet was supplemented with dicalcium phosphate (Ca_2PO_3, containing 21% Ca and 17% P) to achieve the desired calcium concentration according to the treatments. The prawns were fed 4% of body weight, 3 times per day.

Observations of moulting process (premoult, moult, post moult) were conducted continuously during 2 weeks. Timer and stop watch were used to measure the duration of the process. The water hardness was measured at beginning and then every 7 days. Fe-

Table 1. Composition of the experimental diet (% dry weight)

Ingredients	Number
Water (%)	9.21
Ash (%)	7.46
Protein (%)	30.39
Fibre (%)	7.95
Crude fat (%)	10.18
Energy (Kcal/kg)	3844
Calcium (%)	0.82
Phosphorous (%)	0.47

Source:
The proximate analisis was performed by Animal Nutrition and Animal Feed Chemistry, Husbandry faculty of Padjadjaran University

cundity and eggs hatching rate were measured for gravid females observed.

The methods of measurement for each parameter were as follows:

1. Premoult duration

   The premoulting duration was measured from starting point of old eksoskeleton released from the prawn body until totally loss from the body (Greenaway, 1985)

2. Moulting duration

   The moulting duration was measured in minutes for female prawn from the point where the old eksoskeleton was shed, until post moult, when the new carapace hardened, according to methods described by Vijayan & Diwan (1995)

3. Intermoult

   The intermoult time was measured for each individual from each moult until the next moulting (Ismail & New, 2000)

4. Fecundity

   Absolute fecundity was measured as number of total egg in each female prawn (Malecha, 1983). Egg were collected and enumerated by using a weight sampling method and grids on petridish. Egg number was expressed as egg per gram of female (epgf) (Sukadi, 1995)

5. Eggs hatching rate (HR) or larvae produced

   HR measured as:

$$HR = \frac{\text{Hatched eggs}}{\text{Total eggs}} \times 100\%$$

The number of hatched eggs was based on total larvae produced by each female. The berried females with orange eggs from each of the treatments were placed in 50 L hatching jars. Larvae that hatched within 24 h were collected and placed in 5 L buckets. Water temperature was maintained at 28ºC-30ºC using a thermostat heater (Rena, 50 W) and gentle aeration was provided. The eggs were not disinfected throughout the incubation period. The total number of larvae was estimated volumetrically by taking 20 mL samples and manually counting for 3 replicates.

To maintain water quality in the mating tanks, monitoring of water quality was conducted weekly. Water samples from the tanks were collected to measure alkalinity, pH, temperature, dissolved oxygen, total ammonia-nitrogen, nitrite-nitrogen, and hardness, then they were analyzed following standard methods of APHA (2005).

Duration of moulting, fecundity, and egg hatching rate were analysed statistically by analisis of variance (ANOVA) to determine differences among the treatments. When F-values were significant, LSD's were determined.

RESULT AND DISCUSSION

Moulting

Average time of premoult (loss of old carapace), moulting, and intermoult of the prawns are presented in Table 2.

Analysis of the data suggest that the premoult time and moult time are related to calcium content in prawn diet ($P<0.05$), while intermoult time was not ($P>0.05$).

The results show that the treatments had a significant effect on duration of moulting and carapace exchange process. Supplement with Ca_2PO_4 at about 4% (0.82% Ca) in the prawn feed is sufficient for the supply of calcium to create a hard shell for the premating moult of prawn broodstock.

Prawn Egg Performance

The quality of prawn eggs is affected by genetics, size and age of female, nutrition, and environmental factors. In the study, the aver-

Table 2. The time of premoult, moult and intermoult in different dosages of calcium (average ± standard error)

Parameters	Treatments (Ca_2PO_4)				
	0%	2%	4%	6%	8%
Premoult (minutes)	7.25±0.5 a)	6.0±0.82 ab)	5.75±0.96 ab)	5.25±0.5 b)	5.0±0 b)
Moult (minutes)	42±3.5 a)	38±0.95 b)	34±1.29 c)	33±1.5 c)	31±1.5 c)
Intermoult (days)	19.25±0.5 a)	18.75±0.96 a)	19±0.82 a)	18.75±0.5 a)	19±0.82 a)

Note: Values followed the same superscript in one row are not statistically different (P>0.05)

age weight of eggs was about 3.78±0.53 g at 4% dietary inclusion of Ca_2PO_4 to 4.53±0.79 g at 6%. Average absolute fecundity was about 40.0968±4.9825 at 2% dietary inclusion of Ca_2PO_4 to 46.1314±8.4177 at 6%. Average eggs hatching rate was about 26.5±9.9% at 0% dietary inclusion of Ca_2PO_4 to 50.8±10.3% at 8%. The average weight of females, total egg weight, absolute fecundity, average of eggs per g female and hatching rate of prawn egg are presented in Table 3.

Analysis of the egg hatching rate data suggests that the egg prawn hatching rate was related to calcium content in prawn diet (P<0.05), while absolute fecundity was not significantly different among the treatments (P>0.05).

The results show that the treatments had a significant effect on freshwater prawn eggs hatching rate, and supplementing 6% Ca_2PO_4 (1.26% Ca) in the prawn feed is the optimum dosage to improve the prawn eggs hatching rate.

Water Quality

Water quality parameters, including temperature, dissolved oxygen (DO), pH, ammonia (NH_3), nitrite (NO_2), and hardness-$CaCO_3$ during this study are presented in Table 4.

DISCUSSION

Development of sexual maturity of *M. rosenbergii* females involves the physiological processes of moulting, somatic growth, and ovarian development. Optimal environmental conditions and balanced nutrition play a significant role in these processes (Cabrita *et al.*, 2009). This study showed that premoult and moult times were significantly different and inversely correlated with calcium content of prawn feed. The processes occur more quickly with higher calcium dosages in the feed, and are most protracted at the lowest calcium level. Wickins (1976) explained that the moulting duration of crustaceans was influenced by several factors, including temperature, animal size, and feed and availability of specific minerals. Addition of minerals at 1.5 to 3% in the feed in giant freshwater prawn might shorten the moulting duration (Soeyoko, 1992 *in* Satyani, 1996). The addition of minerals to the prawn feed might induce higher hemolymph hyperosmotic pressure, and also materials for building the new cuticle would be more easily available, which could accelerate the moulting prosess (Greenaway, 1985). The variation in time of moulting process at different calcium levels may be due to the changes in metabolic activities of the prawn. Calcium is very important to freshwater prawns in keeping exoskeleton strong. When calcium content in the water (water hardness) and the feed are too low, prawns will take a longer time for the exoskeleton to harden after a moult, and prawns are vulnerable to mechanical damage and predators.

Intermoult duration among different calcium levels in this study was not significantly different. However, the intermoult time was relativelly shorter than that of previous experiments as reported by Satyani (1996) and Cavalli *et al.* (2001). They stated that the intermoult period for 16-25 g adult giant freshwater prawns held in captivity was about 21.4 days, while that of 26.2±5.1 g wild prawn females was about 27.5 days. The shorter duration of intermoult time in our study was expected due to the optimum water quality, especially pH and temperature.

The prawn reproduction performance, both egg number and fecundity, was not effected

Table 3. The average of female weight, total egg weight, absolute fecundity, and hatching rate of prawn egg

Treatments Ca_2PO_4 (%)	Average weight of females g ±SE	Average weight of egg g ±SE	Average egg number per g of egg Pieces ±SE	Average absolute fecundity Pieces ±SE	Average egg number per g female Pieces ±SE	Average HR %
0	30.19±2.8	4.17±1.4	11,2698±1,7015	45,2990±8,9668[a]	1,326. ±306.3	26.5±9.9[a]
2	31.5±1.05	4.22±0.42	9,690.3±481.9	40,0968±4,9825[a]	1,148.8±137.4	36.3±8.9[ab]
4	30.66±1.87	3.78±0.53	11,1468±1,4081	41,6768±3,4168[a]	1,215.1±116.4	42.7±15.1[ab]
6	32.94±3.32	4.53±0.79	10,0207±1,3174	46,1314±8,4177[a]	1,214.7±181.8	49.9±6.2[b]
8	30.43±3.0	4.03±0.47	10,5731±927.5	42,5102±6,2269[a]	1,229.9±98.1	50.8±10.3[b]

Note: Different superscript letters in the same column indicate significant differences (ANOVA, $P<0.05$)

Table 4. Temperature, disolved oxigen (DO), pH, ammonia, nitrite, and $CaCO_3$-hardness in the prawn mating tanks

Treatments Ca_2PO_4	Temperature (°C)	DO mg L^{-1}	pH	NH_3-N mg L^{-1}	NO_2 mg L^{-1}	Hardness mg L^{-1} as $CaCO_3$
0%	28.4-30.1	4.1-6.1	7.3-7.6	0.001-0.05	0.0-0.22	9.0±0.22
2%	28.4-30.0	4.2-5.8	7.3-7.9	0.001-0.05	0.0-0.20	10.2 ±0.6
4%	28.5-30.1	4.2-5.9	7.3-7.6	0.0-0.05	0.0-0.24	11.1 ±0.9
6%	28.4-30.1	4.2-6.1	7.3-7.9	0.001-0.05	0.0-0.22	11.7 ±0.6
8%	28.6-30.1	4.2-5.9	7.3-7.9	0.001-0.05	0.0-0.22	12.8 ±0.9

by calcium levels in the prawn diet. Nevertheless, the number of egg or spawning fecundity in this study, was relatively high compared with some previous studies. The absolute fecundity of 30-33 g prawn female was about 40,096-46,131 or 1.1488-1.3264 eggs per gram of female (epgf). Malecha (1983) indicated that the number of eggs or spawning fecundity of 45 g females averaged 41,002 or around 911 epgf for early-stage eggs. Sukadi (1995) reported egg number per g female (epgf) of younger and smaller sized female (14-18 g) that were reared in several levels of water hardness was about 510-676 epgf. Cavalli *et al.* (2001) reported that the number of egg per spawning event varied considerably between individuals, ranging from 26,587 to 74,775 egg (mean of 46,512±11,220 egg) or 911 epgf.

Hatchability and larvae produced in this study was significantly affected by calcium level in the prawn diet. The hatching rate was positively related with calcium levels ($P<0.05$), from 26.5% in the 0% to 50.8% in the highest calcium level. The egg hatching rate in this study was low when compared with other studies reporting hatching rate of 86% (Cavalli *et al*., 2001). At a hatching rate of 86%, it would be possible to obtain around 1,250 larvae per gram of female body weight. Yan & Bart (2008) stated that in low salinity water the larvae production per g female could reach 693±232 larvae. Law *et al.* (2002) reported that hatching rate of eggs for freshwater prawns at optimum pH level (pH 7) reached 92.22%. The low hatching rate in the current study could be due to factors, including egg quality, sperm quality and handling during observation and moving of the prawns from mating tanks to incubation tanks. Wickins & Beard (1974) *in* Cavalli *et al.* (2001) suggested that egg losses during *invivo* incubation could amount to 31% of the eggs initially deposited in the brood chamber. Egg losses were considered to be partially due to consumption by females, the continual sloughing off of dying egg due to epizootic infestations and to the loose nature of the larger grey egg, which would render them more prone to physical losses.

Water quality parameters including temperature, pH, dissolved oxygen, nitrite, and ammonia were in a range suitable for *M. rosenbergii* development (Boyd & Zimmerman, 2000), while hardness ($CaCO_3$) in all tanks was suboptimal, 30-150 mg/L (Zimmerman, 1988 *in* New & Valenti, 2000; Adhikari *et al.*, 2007). Under such condition of low water-hardness, calcium suplementation in the prawn feed is advisable. Sukadi (1999) reported that a dietary level of calcium around 0.25% provides enough calcium for postlarval prawns, even in waters with a hardness of 18 mg L^{-1}. New (1995) explained that dietary calcium was important for prawns reared in soft water (low water hardness). Further, New (1995) stated that the highest survival, average weight, and biomass were obtained with 3% dietary calcium and 51 mg L^{-1} $CaCO_3$ in the water. Therefore, for successfull prawn mating in the breeding program, especially when conducted in soft water, calcium supplementation of the prawn feed was recommended to improve survival rate and larvae production.

CONCLUSION

There were no significant differences in fecundity and intermoult time for female prawns with different treatments of dietary calcium ($P>0.05$).

Premoult, moult and egg hatching rate were significantly affected by calcium supplementation in the prawn diet. The duration of pre-

moult and moult processes was reduced in a linear fashion ($P<0.05$). Moulting time reduced from 42 minutes to 31 minutes as calcium increased from 0% to 8%. Premoult time reduced from 7.5 minutes to 5 minutes as calcium increased from 0% to 8%. The egg hatching rate increased from 26.5±9.9% - 50.8±10,3% as calcium increased from 0% to 8%.

The results of this study suggested that in minerally soft water (9-12.7 mg/L as $CaCO_3$) calcium supplementation of the prawn feed is required to support the moulting process and egg quality of the giant freshwater prawn.

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