Use of *Spirulina*-enriched Blood Clam (*Anadara granosa*) instead of Sand Worm as Fresh-food Maturation Diet in Black Tiger Shrimp (*Penaeus monodon*) Broodstock

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ABSTRACT

The effect of fresh-food maturation diets on reproductive performance of black tiger shrimp (*Penaeus monodon*) broodstock was compared. Female broodstock shrimp of 10-12 inches in total length and weighing not less than 200 g. and male broodstock shrimp of 8 inches or larger with body weight of not less than 96 g. were used for the experiment. Shrimp were randomly stocked into two 4-m^3 maturation tanks with 19 females and 15 males per tank. Treatment 1, diet A was composed of 20% swimming crab (*Portunus pelagicus*), 10% sand worm (*Perinereis* sp.) and 20% squid, while treatment 2, diet B was composed of 20% swimming crab, 10% *Spirulina*-enriched blood clam (*Anadara granosa*) and 20% squid. The formulation of diet A is the most commonly used for fresh-food feeding by shrimp hatcheries in Thailand. Each treatment of broodstock was fed with different natural food at different percentage of shrimp body weight three times a day (at 0500, 1300 and 2200 hrs) throughout the 30-day feeding trial. At the end of the trial, the reproductive performance of each treatment was compared. The total and average number of spawning of treatment 1 (19 females) was 64 or 3.37±1.12 per female, respectively, compared to 62 or 3.26±1.32 per female, respectively, in treatment 2 (19 females). The number of eggs and nauplii produced from treatment 1 were 68.55 x10^6 and 36.7 x10^6, respectively, compared to 61.20x10^6 and 35.2 x10^6, respectively, for treatment 2. There were no significant differences ($P>0.05$) between treatments 1 and 2. Moreover, percentage hatching rates and metamorphosis to nauplius stage in both treatments were not significantly different ($P>0.05$). However, total carotenoids in eggs from treatment 1 was 3.22±0.10 μg g^{-1} which is significantly lower than 7.82±0.14 μg g^{-1} from treatment 2 ($P<0.05$). This study indicated that *Spirulina*-enriched blood clam could be used as a replacement for sand worm in fresh-food maturation diet for *P. monodon* broodstock if female broodstock size is 10-12 inches in total length.

Key words: *Penaeus monodon*, blood clam, sand worm, maturation diet, *Spirulina* sp.

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INTRODUCTION

Since mid-1980s intensive black tiger shrimp (*Penaeus monodon*) farming have become an important activity in Thailand and other Southeast Asian countries as shrimp became a major export commodity. However, larval production still relies on wild-caught broodstock. The availability and quality of spawners are always affected by various factors such as climate and season. More commonly, large *P. monodon* female broodstock captured from the wild are induced to mature in captivity, after which they would spawn. Induced maturation is generally achieved through a combination of eyestalk ablation, special diets, temperature control, photoperiod and light control, and other manipulations (Beard and Wickins, 1980; Primavera, 1985; Menasveta *et al*., 1993, 1994b). Typical feeding practices for maturation of shrimp broodstock still rely on nutrition derived from a variety of natural marine organisms such as squid, annelid worms (polychaetes), bivalves (mussels, clams and oysters), crustaceans (shrimp, crab, krill, enriched *Artemia*) and fish (Primavera, 1983; Menasveta *et al*., 1993; Browdy,1998; Wouters *et al*., 2001; Coman *et al*.,2007).

Polychaetes particularly sand worms (*Perinereis* sp.) are the main live food extensively used in Thailand as part of the broodstock maturation diet due to their quality in enhancing shrimp reproductive performance (Middleditch *et al*., 1979; Lytle *et al*., 1990). Such success partly results from their highly unsaturated fatty acids (HUFAs) component, particularly arachidonic acid content (Meunpol *et al*., 2005a) as well as some reproductive hormones such as prostaglandin E2 (Meunpol *et al*., 2005b) and prostaglandin F2α (Poltana, 2005). Other hormones discovered in polychaetes are progesterone and estradiol which are important in crustacean reproductive system functions (Fingerman, 1997). However, the major constraint of using sand worms is the uncertain quantity and quality due to seasonal and environmental variations. Moreover, viral disease contamination from sand worms particularly from cultivated areas is a major concern and has been attributed to cause disease outbreaks especially for white spot disease.

Several research work demonstrated that carotenoid is an important nutrient affecting reproductive performance of shrimp, improved embryonic and larval development, cellular protection from photodynamic damage, enhanced growth and maturation, and formation of in-chain epoxides that act as oxygen reserves under anoxic condition (Middleditch *et al*.,1979; Millamena,1989; Bray *et al*.,1990; Torrissen, 1990; Menasveta *et al*.,1994a). Since crustaceans are unable to synthesize carotenoids de novo, appropriate precursors must be supplied in the diet.

Spirulina, a filamentous, blue-green algae, is a rich source of carotenoids especially β-carotene and xanthophylls (Miki *et al*., 1985). Matthew *et al*.(1995) reported that carotenoid content in commercially available *Spirulina* ranges from 3.5 to 5.7 g kg^{-1} and could be used as low-cost carotenoid source for broodstock diets. This present study was conducted to evaluate the use of *Spirulina*-enriched blood clam (*Anadara granosa*) as a fresh-food maturation diet for *P. monodon* broodstock by comparing it to sand worm in terms of number of spawning, fecundity,

hatchability and metamorphosis to nauplius stage. The knowledge gained from this study will be useful for future shrimp maturation programs.

MATERIALS AND METHODS

Broodstock shrimp

The broodstock of *P. monodon* were captured in Andaman Sea, offshore of Satun province, southern Thailand. The shrimp were then transported to Sureerath farm, Chantaburi province and acclimated in two fiberglass tanks (4 m^3) for screening of White Spot Syndrome Virus (WSSV) and Monodon Baculovirus (MBV) prior to size selection. Female broodstock of 10-12 inches in total length and weighing not less than 200 g, and male broodstock shrimp of 8 inches or larger with body weight of not less than 96 g were selected for the experiment. This method of selecting *P. monodon* broodstock is commonly practiced and gave satisfactory results (Limsuwan and Chanratchakool, 2004). Shrimp were randomly stocked into two 4-m^3 maturation tanks with 19 females and 15 males per tank (Table 1). Water quality parameters such as dissolved oxygen (DO), temperature, salinity, conductivity and pH were measured daily using YSI DO 200-4M, while alkalinity, hardness, ammonia and nitrite were analyzed weekly following standard methods by APHA *et al*., 1995.

Table 1. Comparison of the mean (±SD) body length and body weight of brooders used

Parameter	Female	
	Treatment 1	**Treatment 2**
Mean(±SD) body length (inch)	10.86±0.52 [a]	11.46±0.53 [a]
Mean(±SD) body weight (gram)	223.89±24.86 [a]	241.47±17.88 [a]
	Male	
	Treatment 1	**Treatment 2**
Mean(±SD) body length (inch)	8.98±0.48 [a]	8.9±0.35 [a]
Mean(±SD) body weight (gram)	120±23.30 [a]	118.7±12.74 [a]

Remark: Treatment 1 Broodstock fed on 20% swimming crab, 10% sand worm and 20% squid.
Treatment 2 Broodstock fed on 20% swimming crab, 10% *Spirulina*-enriched blood clam and 20% squid.
Average values with the same letter in the same row are not statistically significantly different (P>0.05)

Maturation diets

Two experimental diets (A and B) were formulated with different proportions of fresh-food ingredients. Brooders were fed three times daily (at 0500, 1300 and 2200 hrs) throughout the 30-day experiment.

Treatment 1 Shrimp were fed with diet A: a common combination of fresh-food items containing 20% swimming crab (*Portunus pelagicus*), 10% sand worm (*Perinereis* sp.) and 20% squid (% shrimp body) as shown in table 2.

Treatment 2 Shrimp were fed with diet B containing 20% swimming crab, 10% *Spirulina*-enriched blood clam (*Anadara granosa*) and 20% squid (% shrimp body) as shown in table 2. The blood clam used in this study had been fed with *Spirulina* at 30% of body weight (30 g *Spirulina* for 100g of blood clam) for 3 days before feeding it to the broodstock. *Spirulina* sp. (spray dried powder, food grade, ASB SPIRUMATE, Advanced Spirulina Biotechnology Co., Ltd. Thailand.) contains 70% protein (8 essential amino acids and 10 non-essential amino acids) in correct proportion, 15% carbohydrates, 7% essential fatty acids, 8% vitamins (B1, B2, B3, B6, B12, folic acid, vitamins E and K, inositol, biotin and pantothenic acid) and minerals (calcium, magnesium, potassium, phosphorus, sodium, manganese, zinc, copper, iron and chromium) and pigments.

Table 2. Feeding program of diet A and B for broodstock *P. monodon*

Experimental diet	Feeding time		
	0500 hrs	1300 hrs	2200 hrs
Diet A	swimming crab (20% shrimp body)	sand worm (10% shrimp body)	squid (20% shrimp body)
Diet B	swimming crab (20% shrimp body)	*Spirulina*-enriched blood clam (10% shrimp body)	squid (20% shrimp body)

Reproductive and spawning performance

Female broodstock were examined daily for ovarian maturation by shining a torch beam through the dorsal exoskeleton. Ripe females (ready to spawn) recorded as having stage-4 ovaries (Tan-Fermin and Pudadera,1989) were transferred to spawning tanks (1 m^3) and allowed to spawn. After spawning, females were returned to the maturation tank and the number of eggs per spawning was estimated from the sample of eggs collected between 4h and 8h post-spawning, by taking out four samples of 250 ml of water from the spawning tank. The estimated value was used to calculate fecundity. Eggs were then allowed to hatch in the spawning tanks and the number of nauplii per spawning was estimated from the total number of nauplii collected from four 250 ml samples taken 2 to 5 hrs after first hatching.

Carotenoids analysis

The egg samples from each treatment were collected, re-suspended in 1.5 mL isotonic saline solution (400mM NaCl, pH 7.4), homogenized and centrifuged at 1000g for 10 min. The supernatant was analyzed for total carotenoids following the method described by Regunathan and Wesley (2006).

Data analysis

Number of spawning and eggs, hatching rate, and metamorphosis to nauplius stage from two treatments were calculated and compared statistically using the T-test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

During the 30-day feeding trial, the average number of spawning per female in treatment 1 was 3.37±1.12 while that of treatment 2 was 3.26±1.32 which was not significantly different (P>0.05) from each other. The total number of eggs from treatment 1 and 2 (both 19 females) was $68.55x10^6$ and $61.20x10^6$, respectively. No significant differences were found in the average number of eggs per spawning and per female between the treatments (P>0.05).

There were also no significant differences between treatments in terms of percentage hatching and total number of nauplii (Table 3). Total carotenoid content in eggs from treatment 1 (sand worm) was significantly (P<0.05) lower than in treatment 2 (*Spirulina*-enriched blood clam) (Table 3).

Table 3. Mean ±S.D. reproductive parameter of broodstocks in treatment 1(sand worm) and treatment 2 (*Spirulina*) at stocking of reproductive performance trial

Reproductive parameter	Source	
	Treatment 1	Treatment 2
Number of spawning (19 females)	64	62
Average number of spawning per female	3.37±1.12 [a]	3.26±1.32 [a]
Total number of eggs (19 females)(million)	68.55	61.20
Average number of eggs per spawning (million)	1.06±0.95 [a]	0.98±1.02 [a]
Average number of eggs per female(million)	3.61±1.12 [a]	3.52±1.55 [a]
Successive number of hatching to nauplii	52	59
Successive number of hatching to nauplii (%)	81.25±3.22 [a]	79.05±5.21 [a]
Successive number of hatching to nauplii per female	2.74±0.58 [a]	3.11±0.97 [a]
Hatching rate (%)	53.97±7.85 [a]	50.53±6.21 [a]
Total number of nauplii (million)	36.7	35.2
Average nauplii per female (million)	1.93±1.07 [a]	1.85±0.82 [a]
Total carotenoids in eggs ($\mu g\ g^{-1}$)	3.22±0.10 [a]	7.82±0.14 [b]

Remark: Treatment 1 Broodstock fed on 20% swimming crab, 10% sand worm and 20 % squid.
Treatment 2 Broodstock fed on 20% swimming crab, 10 % *Spirulina*-enriched blood clam and 20 % squid.
Average values with different letter in the same row are statistically significantly different (P<0.05)

The water quality parameters from both treatments during the 30-day of feeding trial are presented in Table 4. There were no significant differences (P>0.05) in water quality parameters in both treatments. Water quality parameters from both treatments were suitable for shrimp broodstock due to high water exchange rate done daily to ensure good water quality for the brooders.

Table 4. Mean ±S.D. for water quality during the feeding experiment in treatment 1 (sand worm) and treatment 2 (*Spirulina*)

Water quality parameter	Treatment 1	Treatment 2
	Mean±S.D.	Mean±S.D.
Temperature (°C)	27.27±0.56 [a]	27.18±0.69 [a]
pH	7.63±0.11 [a]	7.68±0.05 [a]
Salinity (ppt)	32.85±0.24 [a]	33.04±0.31 [a]
Electrical conductivity (mmhos/cm)	50.92±0.32 [a]	51.21±0.48 [a]
Total alkalinity (mg/L)	140.25±7.60 [a]	140.78±12.01 [a]
Hardness (mg/L)	6914.93±43.81 [a]	6955.71±65.66 [a]
Total ammonia nitrogen (mg/L)	0.10±0.08 [a]	0.09±0.08 [a]
Nitrite nitrogen (mg/L)	0.63±0.43 [a]	0.50±0.43 [a]

Remark: Treatment 1 Broodstock fed on 20% swimming crab, 10% sand worm and 20 % squid.
Treatment 2 Broodstock fed on 20% swimming crab, 10 % *Spirulina*-enriched blood clam and 20 % squid.
Average values with different letter in the same row are statistically significantly different (P<0.05)

Concerning the maturation and reproductive biology of the shrimp, the study on broodstock size revealed that larger females (weight >120 g) could undergo gravidity (stage 4 ovarian maturity) and spawning better than the smaller ones (weight <110 g). Large females could re-mature and spawn more frequently than the small ones. The total egg production of large-sized female broodstock was significantly higher than that of the smaller-sized broodstock (Menasveta *et al*., 1994b). In this study, the size of female broodstock in both treatments was quite large (10-12 inches) which was suitable for spawning and gave satisfactory results in terms of number of spawning and eggs (Limsuwan and Chanratchakool, 2004).

Although a wide range of aquatic organisms had been used as feed for shrimp broodstock (Middleditch *et al*., 1979), farmers prefer polychaetes to other live feeds due to their ability to improve reproductive performance of shrimp (Lytle *et al*., 1990). Aside from their high nutrient value,

polychaetes contain some reproductive hormones especially prostaglandin E_2 (PGE_2) which acts as a vitellogenesis-inducing factor for final maturation in shrimp (Meunpol *et al*., 2010). Kawahigashi (1998) reported that marine polychaetes such as *Glycera dibranchiate* and *Americonuphis resii* are the most expensive ingredients used in hatcheries, and maturation operators consider them to be indispensable for stimulation of ovarian maturation. In contrast, our study found that there was no difference between the female broodstock performance in both treatments (treatment 1 – fed with polychates and treatment 2 - fed with *Spirulina*-enriched blood clam) in terms of number of spawning, egg fecundity and hatching rates. This indicates that blood clam enriched with *Spirulina* could be used as an ingredient in fresh maturation diet instead of sand worms.

Spirulina has been found to be a valuable feed as well as a low-cost carotenoid source for shrimp (Tanaka, 1978; Liao *et al*, 1993; Chien and Shiau,1998). Much work have been done to investigate the influence of dietary carotenoids on reproductive performance or larval development of shrimp (Wyban *et al*., 1997; Pangantihon-Kuhlmann *et al*., 1998; Palacios *et al*., 1999; Regunathan and Wesley, 2006). Carotenoids have been suggested to have the capacity to trigger shrimp vitellogenesis, and the effect is directly related to the transcription of hormone genes directly involved in maturation of the ovary (Linan- Cabello *et al*., 2004). Inclusion of carotenoids (astaxanthin) in broodstock diet improved ovarian development and spawning (Pangantihon-Kuhlmann *et al*., 1998; Paibulkichakul *et al*., 2008). Menasveta *et al*. (1994a) reported that greater dietary astaxanthin resulted in greater accumulations of astaxanthin in muscle and ovaries. Other researchers have reported similar results concerning the effects of astaxanthin on ovarian maturation (Pangantihon-Kuhlmann *et al*., 1998; Sagi *et al*., 1996; Paibulkichakul *et al*., 2008; Ribero *et al*., 2001). During ovarian maturation, crustaceans mobilize astaxanthin from the hepatopancreas to ovaries via the hemolymph with lipovitellin in the oocytes (Harrison, 1990). This agrees with our result, where we found that female broodstock fed on blood clam enriched with *Spirulina* resulted in spawning eggs with greater carotenoid content compared with the female shrimp fed on sand worm. This result is similar to the report by Dall *et al*. (1995) which found that the maturing ovaries of *P. esculentus* contained high level of carotenoids. Wyban *et al*. (1997) reported increased nauplii quality with the addition of paprika (as carotenoid source) to the maturation diet. In addition, Palacios *et al*. (2001) reported that larvae with low survival up to Z_3 stage came from eggs with lower carotenoid levels. The results from this experiment make it evident that the broodstock fed on sand worm produced a similar number of eggs and had a similar hatching rate with that of broodstock fed on *Spirulina*-enriched blood clam.

CONCLUSION

In conclusion, this study has shown that it is possible to use *Spirulina*-enriched blood clam as an alternative to sand worm as fresh-food for *P. monodon* broodstock.

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