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Effects of polychaetes (*Perinereis nuntia*) on sperm performance of the domesticated black tiger shrimp (*Penaeus monodon*)

Rungnapa Leelatanawit [a], Umaporn Uawisetwathana [a], Jutatip Khudet [b], Amornpan Klanchui [a], Suwanchai Phomklad [b], Somjai Wongtripop [b], Pacharaporn Angthoung [c], Pikul Jiravanichpaisal [d], Nitsara Karoonuthaisiri [a,*]

[a] Microarray Laboratory, National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency, Klong Luang, Pathum Thani, Thailand
[b] Shrimp Genetic Improvement Center (SGIC), National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency, Surat Thani, Thailand
[c] Program in Biotechnology, Faculty of Science, Chulalongkorn University, Bangkok, Thailand
[d] Aquatic Molecular Genetics and Biotechnology Laboratory, National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency, Klong Luang, Pathum Thani, Thailand

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ABSTRACT

Sand polychaetes (*Perinereis nuntia*) have been used as a live feed to boost reproductive maturation before mating in hatchery farms in Thailand. However, no study has evaluated the effect of polychaetes on performance and physiology of the sperm of the domesticated black tiger shrimp (*Penaues monodon*). Therefore, this study aims to study the effects of sand polychaetes on growth, survival, reproductive performance and sperm physiology and morphology of domesticated male broodstock *P. monodon*. After feeding with either polychaetes or commercial pellets for four weeks, growth and survival rates of polychaete-fed group were significantly higher than those of pellet-fed group. Spermatophore weight and total sperm counts of the polychaete-fed group were significantly higher than those of the pellet-fed group at Weeks 3 and 4, whereas % abnormal sperm cells and % acrosome reaction of polychaete-fed group were significantly lower and higher than those of pellet-fed group only at Week 4, respectively. Physiology of spermatophores in the polychaete-fed group was white and opaque while that of the pellet-fed group was pigmented. Morphological changes of sperm revealed less abnormal sperms (misshaped half, misshaped head, and misshaped tails) in the polychaete-fed group. Nutritional analysis revealed that polychaetes have significantly higher total protein, fat contents and essential fatty acids (arachidonic and eicosapentaenoic acids) but significantly lower fiber content. Moreover, histological analysis of hepatopancreas revealed more vacuoles, which are storages for glycogen and lipids in the polychaete-fed group than in the pellet-fed group. Therefore, this study provides an evidence that polychaetes provide benefits to growth, survival and sperm performance for boosting reproductive maturation in male domesticated broodstock *Penaeus monodon*.

1. Introduction

Sustainability of black tiger shrimp farming is threatened by poor reproductive maturation in captivity. Among various factors contributing to the reproductive maturation, nutrition plays a critical role in promoting reproductive maturation and mating, enhancing fertility, and increasing quality and viability of offspring in crustacean (Harrison, 1990). In penaeid shrimp, natural live feeds (e.g., polychaetes, oysters, squids, clams, mussels, crab, and artemia) have been used to enhance reproductive maturation (Bray and Lawrence, 1992; Browdy, 1998; Peixoto et al., 2005; Rothlisberg, 1998) due to their high levels of polyunsaturated fatty acids (PUFA) such as arachidonic acid (20:4 n-6), eicosapentaenoic acid (20:5 n-3) and docosapentaenoic acid (22:5 n-3), which were reported to be involved in reproductive functions in female (Hoa et al., 2009; Spaziani et al., 1995) and male penaeid shrimp (Braga et al., 2010; Meunpol et al., 2005; Perez-Velazquez et al., 2003; Shailender et al., 2012).

Among the live feeds, polychaetes, either as a single feed diet or as a part of a combination feed, are commonly used for broodstock due to their ability to enhance ovarian maturation (Lytle et al., 1990; Middleditch et al., 1979) due to their high levels of protein, lipid, PUFA, and other hormonally active compounds (Dall et al., 1991; Lytle et al., 1990; Marsden et al., 1997; Middleditch et al., 1979; Naessens et al., 1997). In male shrimps, although the enhancing effects of polychaetes on reproduction of the male shrimp have not been established, it becomes a convenient practice for the shrimp farmers to also use the same feed to boost both male and female broodstock before mating process (Chamberlain and Lawrence, 2009; Lytle et al., 1990).

Previously, knowledge on the effect of polychaetes on male shrimp reproduction is limited because most available literatures examined

* Corresponding author. Tel.: +66 2 5646700; fax: +66 2 5646707.
E-mail address: nitsara.kar@biotec.or.th (N. Karoonuthaisiri).

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effects on a mix of live feeds instead of a single feed. For example, the effect of a mix of live feeds (squid (*Illex argentines*), blue crab (*Callinectes sapidus*), and fish (*Macrodon ancylodon*)) was studied in the male pink shrimp (*Farfantepenaeus paulensis*) (Braga et al., 2010). In the white shrimp (*Litopenaeus vannamei*), effects of marine bloodworms (*Glycera dibranchiata*) mixed with other live feeds on male reproduction were reported (Perez-Velazquez et al., 2003). In the black tiger shrimp *Penaeus monodon*, effects on the domesticated male shrimp of sand polychaetes (*Perinereis* spp.) and mud polychaetes (*Marphysa* spp.) mixed with other live feeds were examined (Meunpol et al., 2005). Another study examined sperm quality of the male domesticated broodstock *P. monodon* fed with the experimental diet containing a similar amount of highly unsaturated fatty acids (HUFA) to that of polychaetes and found no improvement in sperm quality (Meunpol et al., 2005). Although a recent study has compared the effect of live feeds, such as polychaetes (unspecified species), squid (unspecified species) and oyster (unspecified species) on male reproductive maturation of wild *P. monodon* by assessing spermatophore weight, total sperm count, and % of live and abnormal sperm (Shailender et al., 2012), it has not specified the exact species of the polychaetes used in the experiment and has not examined the effects on physiology and morphology of the black tiger shrimp. Moreover, it did not study the effects on the domesticated shrimp. Therefore, this current study aims to examine the efficiency of the sand polychaetes (*Perinereis nuntia*) on improving growth rate, sperm performance, sperm physiology and morphology of the domesticated black tiger shrimp.

2. Materials and methods

2.1. Experimental broodstocks

Domesticated male brooders (17-month-old, Broodstock Multiplication Center, Burapha University, Chanthaburi, Thailand) and the domesticated female brooders (17-month-old, Shrimp Genetic Improvement Center (SGIC), Suratthani, Thailand) were transferred to a commercial hatchery farm (Bunjonk farm, Chachoengsao, Thailand) and acclimatized to the experiment condition for one month prior to the experiment.

2.2. Feed preparation

Commercial pellets used in this experiment were purchased from Charoen Pokphand Foods PCL. (CPF, Thailand, >35% protein, >5% lipid, <12% moisture, and <4% ash), and sand polychaetes (*P. nuntia*) were cultured for 7 months at SGIC by being fed 2 times/day with another commercial feed (CFP, >38% protein, >5% lipid, <11% moisture, and <3% ash). The conditions used to culture the polychaetes mimic the natural habitat in the intertidal zone by transferring seawater out in the evening and adding new seawater in the subsequent morning. These conditions were commonly practiced at the SGIC.

2.3. Feed experiment and sample collection

This experiment was carried out from December 2012 to January 2013 at a commercial hatchery farm (Bunjonk farm, Chachoengsao, Thailand). A total of 382 domesticated male brooders were randomly separated into 8 circular concrete tanks (diameter 4 m, height 1.2 m). Two groups (one fed with commercial pellets and the other fed with polychaetes) were conducted (4 tanks/group; 60–62 shrimp/tank). Each group was fed 17 times/day with either 1.5–2% commercial pellet/shrimp weight/day or 5–6% polychaetes/shrimp weight/day for four weeks. The percentage of feed was determined upon shrimp consumption as previously reported (Millamena and Quinitio, 2000). Seawater 30 ppt was recirculated everyday at a 50% rate and the temperature and pH were maintained at 29.1 ± 0.6 °C and 8.04 ± 0.05, respectively. For each group, a total of 30 shrimps were randomly collected at each time point (before feeding: Week 0; after feeding: Weeks 3 and 4) at the same molting stage (pre-molt stage) to avoid any effect from molting. Molting stages were classified by observing the morphology of shrimp uropod under a light microscopy (Olympus CH30) as previously reported (Promwikorn et al., 2004). Body weight, length, and spermatophore weight of shrimp ($N = 30$ per each group) were recorded at Weeks 0, 3, and 4 and the averaged values for each index were reported. Survival rate was determined from the following equation:

$$\%\ \text{Survival rate} = \frac{\text{Numbers of shrimp at Week 0} - \text{Numbers of dead shrimp}}{\text{Numbers of shrimp at Week 0}} \times 100$$

2.4. Sperm performance assessment

Quality and quantity of spermatozoa were assessed by (1) total sperm count, (2) percentages of abnormal sperm cells, and (3) acrosome reaction (AR). For each shrimp group, a total of 10 randomly selected shrimps per time point was used for total sperm count and abnormal sperm count. For each shrimp, both spermatophores were collected but homogenized separately in a calcium-free sea water solution. Total sperm count was determined using hemacytometer as previously described (Leung-Trujillo and Lawrence, 1987). Briefly, sperm cells in the calcium-free sea water solution were stained with 0.1% trypan blue prepared in calcium-free solution for 10–15 min after which the dead sperms appeared in blue under a light microscopy (Olympus CH30). For abnormal sperm count, morphology of live sperms was examined. The abnormality was classified into different types: missing head, bent or missing spikes, and distorted main body. The counting of total sperm number and abnormal sperm number was repeated with 8 aliquots from a sample (4 aliquots/1 side of spermatophore; 2 spermatophore/sample). Each aliquot was counted five times when sperm number is greater than 100. If sperm number is less than 100, all 25 holes of the middle hemacytometer were counted. The average counts of all aliquots were used to represent the sperm counts for that shrimp. For each group and each time point, the averaged counts from all the shrimps were used.

For AR assessment, a total of 10 randomly selected shrimps per time point was used. Both sides of spermatophores of each shrimp were deposited into the thelycum of female broodstock ($N = 10$ per each feed group). An *in vitro* AR was performed by depositing spermatophores into female thelycum for 3 days. These female brooders were fed with polychaetes before the start of the experiment for one month and cultured in the same condition with the male shrimp but at a separated pond. The thelycum-deposited spermatophores were immediately homogenized in a calcium-free sea water solution. Sperm solution was incubated in egg water (EW) with a ratio of 1:1 for 5 min. EW is seawater collected at the time of spawning of female brooder including the egg-derived inducers of sperm AR as previously described (Alfaro et al., 2007; Kruevaisayawan et al., 2008; Pongtippatee et al., 2007). A number of sperms that undergone AR from each group were measured through the microscopy (Olympus CH30). For the EW preparation, female shrimp exhibiting sign of readiness to spawn was firmly held over a 500-mL beaker and allowed to spawn the eggs into filtered seawater. The eggs were settled to the bottom of the beaker, and then EW, seawater without spawned eggs, was centrifuged at 10,000 *g* for 15 min, at 4 °C to remove any particulates and kept at −80 °C until use.

2.5. Morphology studies of hepatopancreas and testis organs of male domesticated broodstock P. monodon

2.5.1. Histological study

Hepatopancreas (HP) samples ($N = 3$, each feed group) were fixed in Davidson fixative (220 mL of 37% formalin, 115 mL of 100% glacial