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DETERMINE THE COMPETENCE OF DIFFERENT FRESH DIETS TO IMPROVE THE SPERMATOPHORE SUPERIORITY OF GIANT BLACK TIGER SHRIMP, *PENAEUS MONODON* (FABRICIUS, 1798)

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Abstract: The intention of the present study was to assess the effect of fresh natural food on spermatophore quality and analysis by the way of sperm weight, count, viability as well as the proximate analysis of offered food and shrimp brood stock. The experiment was carried out with the three following treatments; fresh squid, polychaete and beef liver up to 5 weeks. All parameters were measured by the starting of 3rd week (15 days) and the end of the (35 days) of the experiment. Spermatophore quality was evaluated by spermatophore weight, sperm count, viability and proximate analysis of the treatment and shrimps. The content of three essential fatty acids, Arachidonic acid (AA), Eicosapentanoic acid (EPA) and Docosahexaenoic acid (DHA) from wild *Penaeus monodon* brood stock were evaluated in comparison with natural diet fed to *P. monodon*. Spermatophore of wild male brood stock contained higher levels of AA than those of artificial fed males. Polychaete has higher proportion of AA to EPA and DHA. At the end of the treatment the sperm viability and count, shrimp and spermatophore weight were significantly different among treatment and control ($p<0.05$). The present study concludes that the fresh squid diet is highly preferred over the diets influence on increasing the spermatophore quality, therefore use of fresh squid only is recommended for the maturation of male *P. monodon* brood stock.

Key words: *Penaeus monodon*, Brood Stock, Spermatophore, Diet, Quality, HUFA.

INTRODUCTION

Marine shrimp farming is the most important aquaculture sector in the world and as reported by MPEDA (2011) about 75% of farmed shrimp is produced in Asia where China, India, Malaysia, Thailand and Indonesia. Indonesia serves as the top producers. Thailand is the largest exporter of farmed shrimp in the worlds. In penaeid prawn hatcheries, efforts to ensure the reproductive success of the prawn broodstock have focused almost entirely on the maturation stage of female spwanners. Despite improvements in this area, prawn larval quality is declining; indicating that quality of male spawners may also contribute to reproductive success. Production of high quality larvae and post larvae for use in aquaculture depends on brood stock are also susceptible to fertility problems that could compromise with larval production as reported by Alfaro (1993). Food is an important factor for the sexual maturation as reported Browdy (1992) and male's reproductive performance (Meunpol, *et al.,* 2005). Shrimp fed with impaired reproductive performance which can completely inhabit the reproductive performance which can completely inhabit the reproduction process (Bray and Lawrence, 1992, 1998; Wouters *et al.,* 2001, 2002; Memon *et al.,* 2012). In males spermatophore quality is a tool to evaluate efficiency of the diet to promote maturation and reproduction (Prez-Velazquez et al., 2002, 2003; Meunpol *et al.,* 2005; Coman *et al.,* 2007). Relation with quality of shrimp and spermatophore has also been reported by Coman *et al.,* (2006). P. monodon was found to feed mainly on crustacean and vegetable matters (Hall, 1962; Tiews *et al.,* 1972; Memon *et al.,* 2012).

In Australia, the shrimp is known to consume the remains of small animals and a large amount of unrecognizable material (Dall, 1968). However, studies on the diet of other penaied prawns indicate that a wide range of food may be consumed (Gopalakrishanan, 1952). Moreover in routine, penaeid maturation success in captivity is obtained using diets that include fresh food with or without addition of commercial diet (Browdy, 1992; Harrison, 1990, 1997; Peixoto, *et al.,* 2005). A number of studies have shown that male prawn quality is crucial to successful spawning. Researchers have reported that without good quality sperm, inferior quality of eggs, fertilization rate and larval production will result (Beard and Wickins 1980; Aquacop 1983; Sasikala and Subramoniam1987). Reduction in reproductive performance of male prawn results from a number of reasons that including stocking density of the animals in captivity for a long period of time (Chamberlain *et al.* 1983; Watanable *et al.,* 1984; Leung-Trujillo and Lawrence 1987) and or inappropriate maturation diets. Several reports have suggested that nutrition is the prime factor affecting the quality of sperm (Adiyodi 1985; Samuel *et al.* 1999). Normally, prawn broodstock diets are based on natural foods, e.g., molluscs, squids, clams, mussels, other crustaceans and especially polychaetes and squid (Bray and Lawrence 1992, 1998; Rothlishberg, 1998). Some natural foods have disadvantage such as price fluctuations, unstable nutritional valued, enhanced risks of pathogen transmission and the potential to cause to determination of water quality in the maturation systems (Harrison, 1990; 1997). To offer commercial diet during maturation, the production results compared with fresh food are unsatisfactory (Wouters *et al.,* 2002). A prominent characteristic of these live feeds is a high level of long chain fatty acids, i.e. n-3 and n-6 highly unsaturated fatty acids (HUFAs) (Chamberlain and Lawrence 1981). Highly unsaturated fatty acids are involved in Aquaculture Research development of the reproductive and neuronal systems, bio-membrane production, steroid hormone synthesis and being yolk constituents (Harrison1990). There is a great lack of studies regarding the broodstock management as well as effects of food on reproductive performance of male brood stock of *P.monodon*. Seed production is still largely depends on wild brood stock which can be unpredictable in terms of their quality and quantity. The quality of sperm or spermatophore in mature males was often related directly to hatchery holding time as reported by Aiken and Waddy (1980). Therefore, this study aimed to evaluate the effects of fresh natural foods on spermatophore quality and analysis by the way of sperm weight, count, viability as well as the proximate analysis of offered food and shrimp brood stock with a hope that in future the best quality spermatophore could be utilized for further cryopreservation study.

MATERILAS AND METHODS

Sexually matured *P. monodon* male animals were collected from Kakinada and Vishakhapatnam coast, Andhra Pradesh, India. A total of 50 males for each treatment with mean body weight (BW) of 61.58 ±2.5 to 69.32±1.5g and mean total length (TL) of 20.1±0.5cm were used throughout the study. They immediately transport to the experimental hatchery at Vijayawada in an aerated condition. Precautions were taken to reduce the external stress to the brood stocks by providing ambient environmental conditions during transportation. The present study was conducted to observe the effect of diet on the spermatophore quality of *P.monodon* offered up to 5 weeks. Three fresh diets used were squid, polychaete, and Oyster for all shrimp were divided into here groups for dietetic treatment. Each dietary treatment had three replications (total nine tanks). Wild caught shrimp provided with no supplementary feed in a tank was considered as control. Each holding tanks had density of ten individually in 5.0 m^2 rectangular tanks with water depth of 70 cm. samples were kept in sea water of salinity 30±2 ppt, pH; 7.8±1.8, D.O; 5±0.5 ppm. The temperature was in the range of 27±2.5°C. Specimens were maintained in hatchery condition under normal local photoperiod cycles of about 10 h light; 14 h dark. Feeding was commenced thrice; morning (9.00) afternoon (16.00) and night (22.00) with fresh squid, oyster and polychaete at a rate of 10-20% of the biomass. Left over foods and feces were siphoned out and water exchange was done at about 100% on alternate days. Spermatophore quality was measured by spermatophore weight, sperm count and viability. All parameters were measured by the starting of 3rd week and at the end of the 5th week of the experiment. Total 10 males from each treatment were evaluated. Both spermatophores from each male were extruded manually (Middleditch *et al*., 1979). At the end of the experiment, the whole of remaining shrimp selected from each treatment was sampled. All biological materials were maintained at -20°C until proximate analysis.

Statistical analysis: Data were analyzed using the software package SPSS 16.0 two way of Variance (ANOVA). Pearson correlation (2 tailed) and statistic analysis were executed means were compared with LSD and Duncan *post hoc* test (p<0.5). The parameters measured were used to identify significant differences among the mean value of each parameter, viability was recorded and also the sperm count of different fed group were recorded separately . Percentage of viable sperm was calculated by following equation:

$$\% \text{ of live sperm} = \frac{\text{Observer number of sperms}}{\text{Total number of sperms observed}} \times 100$$

RESULTS

Spermatophore quality analysis:

Viability of sperm: The effects of different fresh dietary treatments on spermatophore quality are shown in Table 1. Fresh dietary treatment significantly increased the viability, counting and motility of sperms. At the end of treatment, squid gave highest sperm viability (95.36±1.82%) it increased about 42.53% as compared to the control (54.80±2.19%). Another treatment (polychaete) also increased the survival/viability of sperm by 69.37±0.76% which was decreased about 27.25% when fed with polychaete compared to squid. But the oyster treatment decreased the viability of the sperm by 55.35±1.82% which was about 20.21% as compared to the polychaete (p<0.05) (Table.1). Effect of different diet on survival or viability of the sperm during treatment is shown in Table no- 1.

Sperm counting: To check the count and maturity of sperm, the shrimp was treated with fresh diet of polychaete, squid, oyster and control (wild). The results in the final weeks treatment (5th week), showed that all shrimps were fully mature and no male was found as without spermatophore. Beside this, the mean count of sperm of Polychaete, squid, oyster and control were recorded as 35.69, 39.47, and 32.48 million respectively.

Table No-1: Viability of sperms in different feed treatments

Weeks	Polychaete	Squid	Oyster	Control
2	62.3 ±1.16	71.28± 1.32	35.12 ±0.62	54.80±2.16
3	64.12±2.24	75.62 ±0.89	42.16±1.24	54.80±2.17
4	66.24±3.02	85.47 ±1.34	53.24±2.80	54.80 ±2.18
5	69.37±0.76	95.36 ±1.82	55.35±1.82	54.80±2.19

Table No-2: Total sperm count in different feed treatment

Particulars	Polychaete	Squid	Oyster	Control
Stock (Prawns)	50	50	50	50
Sample (Prawns)	15	15	15	15
% of survival	100	100	100	100
Initial Weight (g)	65.24±1.6	69.32±1.5	61.58 ±2.5	62.54±2.6
Final Weight (g)	75.41±3.6	78.65±3.8	68.25±1.9	60.25± 2.7
Sperm sac weight (g)	0.1062	0.1221	0.9762	0.1091
Total Sperm (x 10^6)	35.69	39.47	32.48	29.87
% of live sperm	82.64	92.57	76.25	72.54
% of abnormal sperm	32.16	28.74	26.24	38.67

Table No- 3: % of HUFA in different treatment for sperm development

HUFA	Polychaete	Squid	Oyster
AA	7.65 ±0.05	5.35 ±0.6	0.75 ±0.54
EPA	7.54 ±0.22	9.12 ±0.6	1.48 ±0.70
DHA	2.34± 0.64	12.39± 1.5	0.75 ±0.02

Spermatophore weight: In all treatments, the mean of spermatophore weight was significantly higher at the end of experiment period. Comparison between the sperm weight of polychaete, squid, oyster and control found as 0.106±0.004, 0.1221±0.006 and 0.9762±0.0007 mg respectively (Table no-2).

HUFA content: Squid contained the highest HUFA level among natural feeds and DHA was predominant. A lower level of HUFA was found in Oyster and EPA (1.48) was higher than AA (0.75) and DHA (1.48). Polychaete contained a higher level of AA (7.65) than EPA (7.54) and contained almost less amount of DHA. In Squid DHA (12.39) value is higher than AA (5.35) and EPA (9.12).Comparatively highest HUFA values are found in squid competed to Polychaete and Oysters (Table no-3)

DISCUSSION

According to Sasikala and Subramoniam (1987) reported that the quality of male prawn broodstock is as essential as quality of females in prawn larval production, less attention has been paid to male reproductive performance. Recently, the problem of declining prawn quality and quantity in nature has alarmed aqua culturists. Pond rearing of *P. monodon* is one potential approach toward solving the problem of declining quality in wild brood stocks. Although farmed prawns tend to display inferior reproductive quality compared with wild prawns (Menasveta *et al.* 1993), their quality can be improved by several factors, including diet (Brown *et al.* 1979; Adiyodi 1985; Harrison 1990). Good spawning results from both male and female *P. setiferus* broodstock when annelid was used as a dietary supplement. They assumed that success stemmed from the lipid content of this diet Middleditch and colleagues (1979). Similarly, experiments by Perez-Velazquez (2003) on male *P. vannamei* have demonstrated that a composite diet of pelleted feed with either squid or polychaetes improved certain male reproductive characteristics (e.g. spermatophore regeneration time) over a diet of pelleted feed alone. The reproductive performance of male *Macrobrachium malcolmsonii* broodstock can also be enhanced by a combination of natural feed and practical diets (Samuel *et al,* 1999). Maturation diets used for prawn spawners are generally a mixture of squid, horse mussels, oysters and polychaetes. These organisms are rich in HUFA and phospholipids (Chamberlain and Lawrence 1981; Lytle *et al.* 1990). In Thailand, the most common polychaetes used by black tiger prawn hatcheries are sand polychaetes (*Perinereis* sp.) and mud polychaetes (*Marphysa* sp.). Both possess a greater amount of AA than EPA or DHA. In the present study, sand polychaete displayed an even higher proportion of n-6 HUFA than mud Polychaetes. This is in agreement with Pinon (2000) who reported that AA, rather than DHA, was the preferred HUFA in Nereis virens, which found a higher ratio of n-3/n-6 in sand Polychaetes. This difference in polychaete HUFA profiles may stem from differences in habitat, environment and/or the food webs, which the Polychaetes inhabit. In aquaculture, n-3 and n-6 HUFA levels have been linked to broodstock reproductive performance, whereas specific C20 and C22 HUFA have not. Our study is the first to formulate and test on natural diet for prawns with specific fatty acids at specific amounts calculated from natural feeds for prawn broodstock. Gomez and Honculada-Primavera (1993) reported that eye ablation could increase the number of sperm without any effect on sperm sac weight. The sperm sac is amass tissue composed of not only sperm cells but several components like intermediate layers inside spermatophore granules inclusions and other acellular processes (Koda-Cisco and Talbot, 1982). After feeding the treatment diets for a month, no reproductive performance differences were found in males of all groups, except for a higher percentage of abnormal sperm in the experimental diet only treatment. This suggests that it is possible to stock prawns in concrete tanks for up to 1 month without loss of quality, as long as a suitable maturation diet is Broodstock diet for male prawn based on fatty acid content provided. Our findings are in agreement with Pratoomchat (1991), whereas other studies (Chamberlain *et al.* 1983; Bray *et al.,* 1985; Leung-Trujillo and Lawrence1987; Talbot *et al.,* 1989) reported abnormalities in the sperm of *P. monodon* after stocking for longer than 2 weeks in captivity. However, the sperm quality of male *M. malcolmsonii* and *P. vannamai* can be enhanced by supplementing feed pellets with natural feeds (Alfaro and Lozano 1980, 1993; Samuel *et al.,* 1999). It was found in this study that AA, not DHA, was the preferred fatty acid in *P. monodon* male reproductive tissues. Prawns with prominent AA levels in their spermatophores displayed a higher number of total sperm. The role of AA in crustacean spermatogenesis has never been investigated, but its role on fish testosterone production has been well established. According to Peixoto *et al.,* (2005) European sea bass (*Dicentrachus labrax*) spermatophores with an elevated percentage of AA showed a higher fertilization success rate compared with controls. A successful maturation diet not only contains essential nutrients, but also contains the appropriate proportion of specific nutrients, as an imbalance of fatty acids within the diet can have negative effects on reproduction. Males fed the experimental diet had a higher sperm count, but also possessed a greater percentage of abnormal sperms and a lower percentage of live sperm. This may indicate that the HUFA concentrations calculated from polychaetes in this study might not yet represent the most suitable concentrations of AA, DHA and/or EPA in prawn maturation diets.

Good reproductive performance is observed when prawns are fed a mixture of natural feeds and pelleted feeds (Chamberlain and Lawrence 1981; Alfaro and Lozano, 1993; Bray and Lawrence 1998; Samuel *et al.,* 1999). In the present study, varieties of foods have been used to achieve the ideal success for gonadal maturation. The finding indicated that fresh squid treatment was necessary for the gonadal maturation. Browdy (1998) and Peixoto *et al.,* (2005) reported that the variety of fresh food has guaranteed the success of penaied maturation in captivity. However, the effects of various diets in maturation system of spermatophore quality of *P.monodon* are currently in progress. It was well documented that organic compounds in penaeid, dietary lipids and protein were involved in gonadal maturation process (Marsden *et al.,* 1997; Samuel *et al.,* 1999; Wouters *et al.,* 2002; Perez-Velazquez *et al.,* 2003; Meunpol *et al.,* 2005; Coman *et al.,* 2007; Memon *et al.,* 2012). These compounds are the most abundant in foods are used in biosynthesis and mobilized during maturation (Harrison, 1990). In the present study it ie observed that spermatophore weight has no significant difference among the treatments which is well correspond with previous study by Nakayama *et al.,* (2008). Leng-Trujillo and Lawrence (1987) and Alfaro (1993) have reported that the post harvesting process together with handling procedure may produce stress to the brood stocks which would reflect in quality of the spermatophore. However, in present study, there was no decrease of spermatophore weight recorded even at the end of the experiment, possibly due to ideal management of shrimp brood stock in captivity. Spermatophore renewal was completed in every 2 or 3 weeks (Pascual *et al.,* 1998). However, in this study spermatophore of *P.monodon* takes 3-4 weeks time to complete maturation. This period may be decreased by proper captivity conditions like nutritional factors and desired physiochemical parameters (Leung-Trujillo and Lawrence, 1987; Ceballos-Vazquez *et al.,* 2004; Mercier *et al.,* 2006; Memon *et al.,* 2012). It was well documented that the food is an important factor for the food is an important factor for the sexual maturation of gametes and improves male reproduction performance (Dall *et al.,* 1991; Browdy, 1992; Meunpol *et al.,* 2005). Hence, it postulated that food might have a considerable role in the spermatophore maturation. According to Gomez-Honculada-Primavera (1993) sperm sac weight does not deflect sperm maturation and they are also found that eye stalk ablation could increase the number of sperm without any effect on sperm sac weight. The sperm sac is a mass of tissue composed of not only sperm cells several compounds like intermediate layers inside spermatophore granules inclusions and other acellular process (Koda-Cisco and Talbot, 1982). Addition of fresh food items during the maturation of *P.monodon* male is important to reduce the effect of natural spermatophore degeneration. The fresh feed diet can be used without reproduction disruption in males as shown in previous studies with other species of penaeid (Browdy, 1992, 1998; Harrison, 1997; Peixoto *et al.,* 2005; Memon *et al.,* 2012). The highest percentage of normal sperm in relation to body weight and age was reported of normal sperm in relation to body weight and age was reported by Alfaro (1993). At the early few numbers of matured sperms observed (Wang *et al.,* 1995; Perez *et al.,* 2000; Racotta *et al.,* 2003). Similar observation was also reflected in present study. Interestingly, it was recorded *P.monodon* produces fully matured sperms at 5^{th} week of dietary treatment (Table-1).

CONCLUSION

In the present study revealed that fresh squid diet is highly favored over diets due to its higher influence on increasing the spermatophore quality. Hence, it is suggested that the matured sperms produced from black tiger shrimp, *P. monodon* fed with squid can be effectively utilized for further artificial insemination as well as for cryopreservation process.

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