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To Determine the Efficiency of Different Fresh Diets in Improving the Spermatophore Quality of Banana Shrimp *Penaeus merguiensis* (De Man, 1888)

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Abstract: The objective of the present study was 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 broodstock. The experiment was carried out with the three following treatments: fresh squid, polychaete and cockle up to 6 weeks. All parameters were measured by the starting of 3rd week (14 days) and at the end of 6th week (45 days) of the experiment. Spermatophore quality was evaluated by spermatophore weight, sperm count, viability and proximate analysis of the treatments and shrimps. Proximate analysis was performed by method described in AOAC in 1995 and spermatophore count and viability was determined by sperm suspension using modified eosin-nigrosin staining method. At the end of treatments the sperm viability and count, shrimp and spermatophore weight were significantly different among treatments and control ($p<0.05$). Lipids content in squid was 8.52±0.13% and it was significantly higher by 102.8% than cockle (4.2±0.3%). Whereas, polychaete with 6.86±0.07% which was 63.3% higher than cockle. Also, squid fed to shrimp had the highest level lipids (3.3±0.15%), it was significantly higher about 17.85% as compared to the control (2.8±0.15%). The present study concludes that the fresh squid diet is highly preferred over other diets due to its higher influence on increasing the spermatophore quality, therefore use of fresh squid only is recommended for the maturation of male *P. merguiensis* broodstock.

Key words: *Penaeus merguiensis*, banana shrimp, broodstock, spermatophore, diet, quality, improvement

INTRODUCTION

Marine shrimp farming is the most important aquaculture sector in the world and as reported by FIGIS (2007) 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 world. The other 25% is produced mainly in Latin America where Brazil is the largest producer. In 2009, the Malaysian total shrimp production was 69, 241 tons for which white shrimp contributed about 52, 926 tons (DOF, 2010). Production of high quality larvae and post-larvae for use in aquaculture depends on broodstock condition (Racotta *et al*., 2003). The penaeid male's broodstock 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 by Browdy (1992) and male's reproductive performance (Meunpol *et al*., 2005). Shrimp fed with unbalanced or incomplete diets have impaired reproductive performance which can completely inhibit the reproduction process (Bray and Lawrence, 1992; Wouters *et al*., 2001). In males, spermatophore quality is a tool to evaluate efficiency of the diet to promote maturation and reproduction (Perez-Velazquez *et al*., 2002; Meunpol *et al*., 2005; Coman *et al*., 2007). Relation with quality of shrimp and spermatophore have also been reported by Coman *et al*. (2006). *P. merguiensis* was found to feed mainly on crustaceans and vegetable matters (Hall, 1962). Whereas in the Manila and San Miguel Bays of the Philippines, *P. merguiensis* feeds mainly on phytoplankton and benthonic foraminifera's (Tiews *et al*., 1972). In Australia, the shrimp is known to consume the

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remains of small animals and a large amount of unrecognizable material (Dall, 1968). However, studies on the diet of other penaeid prawns indicate that a wide range of food may be consumed (Gopalakrishnan, 1952). Moreover in routine, penaeid maturation success in captivity is obtained using diets that include fresh foods with or without addition of commercial diet (Browdy, 1992; 1998; Harrison, 1997; Peixoto *et al*., 2005).

The most used food items are squid, molluscs (oyster and mussel), crustaceans (shrimp, crab and Artemia), fishes and marine polychaete (Bray and Lawrence, 1992; Rothlisberg, 1998). Some natural foods have disadvantages such as price fluctuations, unstable nutritional value, enhanced risks of pathogen transmission and the potential to cause deterioration 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).

There is a great lack of studies regarding the broodstock management as well as effects of food on reproductive performance of male broodstock of *P. merguiensis*. Seed production is still largely dependent on wild broodstock which can be unpredictable in terms of their quality and quantity. The quality of sperm/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 broodstock with a hope that in future the best quality spermatophore could be utilized for further cryopreservation study.

MATERIALS AND METHODS

Source of animals: Sexually matured *P. merguiensis* male specimens were collected from Kota Kuala Muda, Palau Sayak, Kedah, Malaysia (5°39"N; 100°19"E). A total of 100 males with mean Body Weight (BW) of 24.2±3.84-27.7±0.58 g and mean Total Length (TL) of 15.1±0.5 cm were used throughout the study. They immediately transported to the marine hatchery at the Institute of Tropical Aquaculture, University Malaysia Terengganu (UMT) in an aerated condition. Precautions were taken to reduce the external stress to the brood stocks by providing ambient environmental conditions during transportation.

To determine the efficiency of different diets: Experiment to evaluate the effects of diet on the spermatophore quality of *P. merguiensis* offered up to 6 weeks. Three fresh diets used were squid (*Loligo vulgaris*), polychaete (*Sabellaria* sp.) and cockle (*Anadara granosa*). All shrimps were divided into three 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 tank had density of ten individuals in 3.5 m^2 rectangular tanks with water depth of 65 cm. Samples were kept in sea water of salinity 30±2 ppt, pH: 7.5±0.2, DO: 5±0.06 ppm (or mg mL^{-1}). The temperature was in the range of 27±3°C. Specimens were maintained in hatchery condition under normal local photoperiod cycles of about 13 h light: 11 h dark. Feeding was commenced twice: morning (08:00) and afternoon (16:00) with, fresh squid, cockle and polychaete at a rate of 20-30% of the biomass. Leftover foods and feces were siphoned out and water exchange was done at about 30% on alternate day.

Spermatophore quality and sampling: Spermatophore quality was measured by spermatophore weight, sperm count and viability. All parameters were measured by the starting of 3rd week (14 days) and at the end of 6th week (45 days) of the experiment. Total eight males from each treatment were evaluated. Both Spermatophores from each male were extruded manually (Petersen *et al*., 1996). 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.

Proximate analysis: The concentrations of biochemical components have been using as nutritional and reproductive indicators (Mercier *et al*., 2006). Proximate analysis; moisture, ash, total crude lipid and total crude protein were carried out in squid, polychaete, cockle as well as broodstock. All samples were dried and powdered for direct proximate analyses. However, proximate analysis methods described as estimation of moisture content by the Method of AOAC (1995), crude protein by Micro Kjeldahl Method (AOAC, 1995), crude lipid by Soxhelt Method (AOAC, 1995) and ash according to AOAC (1995).

Statistical analysis: Data were analyzed using the software package MSTAT C program, two way of Variance (ANOVA). Pearson correlation (2 tailed) and statistical analyses were executed means were compared with LSD and Duncan *post hoc* test ($p<0.05$). The factors involved were CRD; different time duration (weeks 3-6), different treatment fed groups fresh (polychaete, squid, cockle and control). The parameters measured were used to identify significant differences among the mean value of each parameter; viability recorded during different weeks of diet, percentage of moisture food and shrimp (dry weight basis), percentage of ash food and shrimp (dry weight basis), percentage of protein food and shrimp (dry weight basis), percentage of lipids food and shrimp (dry weight basis) and sperm count of different fed groups. Percentages of viable sperm were calculated by following equation:

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

RESULTS

Spermatophore quality analysis

Viability mean of different treatments: The effects of different fresh dietary treatments on spermatophore quality are shown in Fig. 1. Fresh dietary treatment significantly increased the viability, counting and motility of sperms. At the end of treatment, squid gave highest sperm viability (88.5±9.25%) it increased about 34.29% as compared to the control (65.95±3.3%). Another treatment also increased the survival/viability of sperm by 68.3±5.16% which was increased about 3.6% when fed with polychaete. But the cockle treatment decreased the viability of the sperm by 52.7±5.8% which was about 25.04% as compared to the control ($p<0.05$).

Overall viability mean recorded in different weeks: The difference of survival/viability of sperm as per time duration start from 3rd week up to 6th week end of the treatments was recorded as 62.14±11.42, 67.6±12.25, 70.7±15.82 and 75.04±14.8% in which sperm viability increased significantly ($p<0.05$) with the increased time duration (weeks) (Fig. 2).

Effect of different diet on viability of the sperm: Effect of different diet on survival/viability of the sperm during treatment are shown in Table 1. The highest value was achieved from squid as 98.1±1.9% at 6th weeks of treatment. Then, polychaete at 6th week achieved similar result (75.5±1.57%) as 3rd week of squid (75.5±1.4%). There was no significant difference observed in control (66.0±3.3%) during the time duration (6 weeks). Moreover, cockle also had not shown significant difference in week 4rth and 5th 52.1±2.1 and 53.1±1.8%, respectively.

Sperm counting analysis: To check the count and maturity of sperm, the shrimp was treated with fresh diet of polychaete, squid, cockle and control (wild). The results in the final weeks treatment (6th 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, cockle and control were recorded as 3.8, 4.9, 3.0 and 3.3 million, respectively (Fig. 3).

Table 1: Viability percentages of sperms recorded at 3rd-6th week, during different treatments polychaete, squid, cockle and control, LSD recorded = 1.156

	Weeks			
Treatments	3	4	5	6
Polychaete	61.95±2.16^{g}	67. 3±1.49^{ef}	68.55±0.91^{e}	75.47±1.57^{d}
Squid	75.5±1.40^{d}	85.20±1.60^{c}	95.20±1.70^{b}	98.07±1.90^{a}
Cockle	45.15±0.5^{i}	52.10±2.10^{h}	53.10±1.80^{h}	60.67±0.60^{g}
Control	65.95±3.3^{f}	65.95±3.30^{f}	65.95±3.30^{f}	65.95±3.30^{f}

Different letters indicate significant difference among the treatments ($p<0.05$)

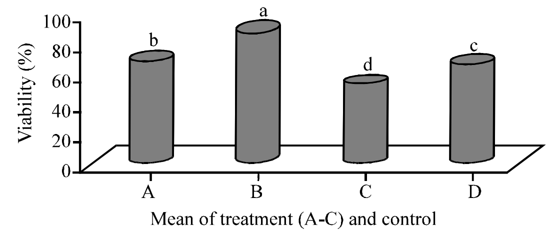

Fig. 1: Total viability mean of different treatments A (68.3) = polychaete, B (88.5) = squid, C (52.7) = cockle and D (66.0) = control at the end of experiment (n = 57); different letters indicate significant difference among the treatments ($p<0.05$)

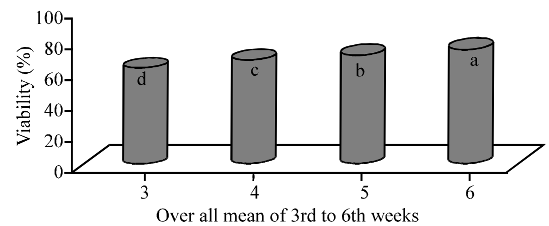

Fig. 2: Overall mean of 3rd-6th (62.14, 67.61, 70.70, 75.04) weeks (n = 57); different letters indicate significant difference among weeks ($p<0.05$)

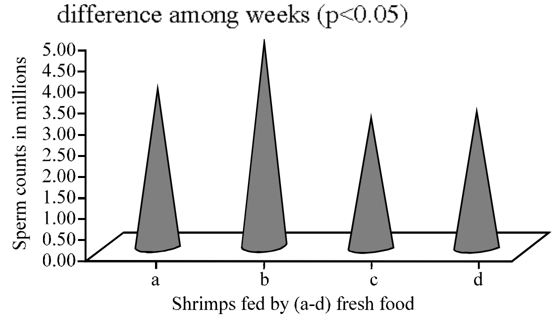

Fig. 3: Parameters of sperm count of the *P. merguiensis* at 6th week, fed fresh foods a (3.78) = Polychaete, b (4.91) = Squid, c (3.04) = Cockle and d (3.30) = control (n = 57); counting method followed by David *et al.* (2009)

Spermatophore weight analysis: In all treatments, the mean of spermatophore weight was significantly higher at

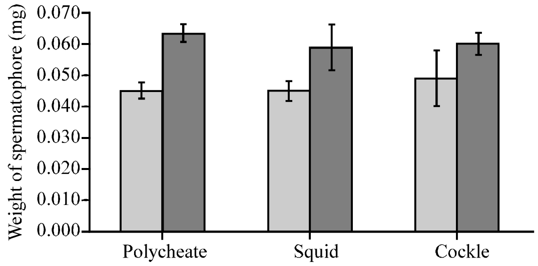

Fig. 4: Comparison of 3rd and last week spermatophore weight. Values are mean±SE (n = 57); 1st count (Polycheate = 0.045, Squid = 0.045, Cockle = 0.049); last count (Polycheate = 0.064, Squid = 0.059, Cockle = 0.060)

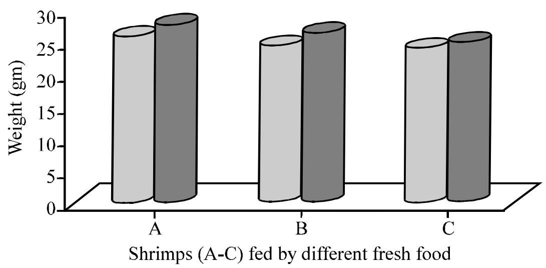

Fig. 5: Weight gain during 6 weeks in different diets. Weight a (A = 25.8, B = 24.6, C = 24.2) = Initial weight and weight b (A = 27.7, B = 26.3, C = 25) = Weight end of the treatment (n = 57); Treatments groups, A = Polychaete, B = Squid, C = Cockle

the end of experimental period. Comparison between the sperm weight in 3rd and 6th weeks was done. In 3rd week, polychaete, squid and cockle was recorded as 0.045±0.002, 0.045±0.003 and 0.049±0.009 mg, respectively and for 6th week, 0.064±0.003, 0.059±0.007 and 0.060±0.004 mg, respectively (Fig. 4).

Shrimp weight gain (SGR) analysis: The weights of shrimp before starting of the treatment were 25.8±0.41, 24.6±2.72 and 24.2±3.84 g for polychaete, squid and cockle, respectively. However, at the end of 6th week, it increased to 27.7±0.58, 26.3±4.04 and 25±4.36 g for polychaete, squid and cockle, respectively. The weight increment percentages were 7.4, 6.9 and 3.3% for polychaete, squid and cockle, respectively (Fig. 5 and 6).

Proximate analysis of offered foodstuff: The percentages of moisture, ash, protein and lipids of fresh diets which offered to shrimps were analyzed on dry weight basis are given as under:

Moisture content in offered fresh diets: Polychaete had the highest level of moisture (14.5±0.4%) it was

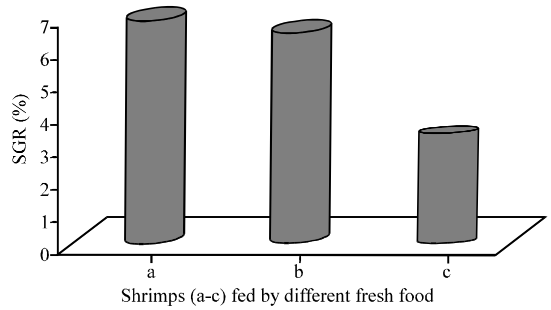

Fig. 6: Weight increment percentages during 6 weeks treatment. Values are mean±SE (n = 57); Treatments groups, a (6.85) = Polychaete, b (6.46) = Squid, c (3.2) = Cockle

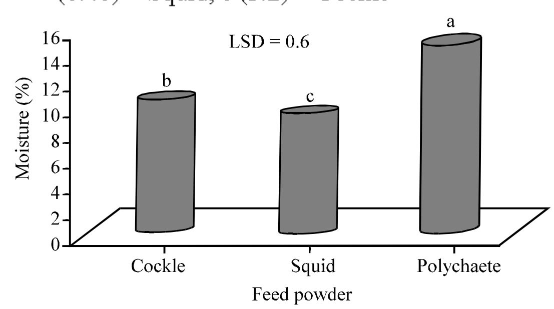

Fig. 7: Moisture content of offered different fresh food to the shrimps (n = 43); different letters indicate significant difference among treatments ($p<0.05$); Cockle = 10.04; Squid = 8.79; Polychaete = (14.45)

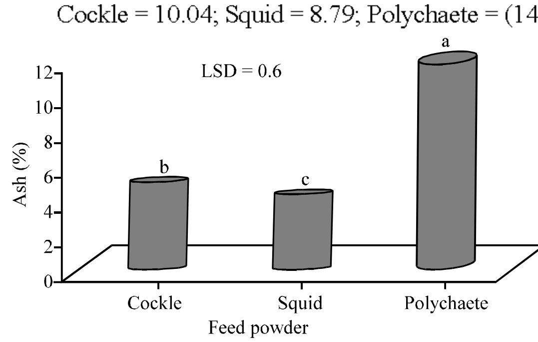

Fig. 8: Ash content of offered different fresh food to the shrimps (n = 43); different letters indicate significant difference among treatments ($p<0.05$); Cockle = 4.59; Squid = 3.83; Polychaete = 11.70

significantly higher about 61.1% then squid (8.9±0.4%). Followed by, cockle (10.04±0.07%) which was also higher then squid about 11.9% (Fig. 7).

Ash content in offered fresh diets: Ash content of polychaete was recorded as 11.7±0.4%; it was significantly higher about 207.8% than squid (3.8±0.1%). Whereas cockle had 4.6±0.1%, this was higher than squid by 21.05% (Fig. 8).

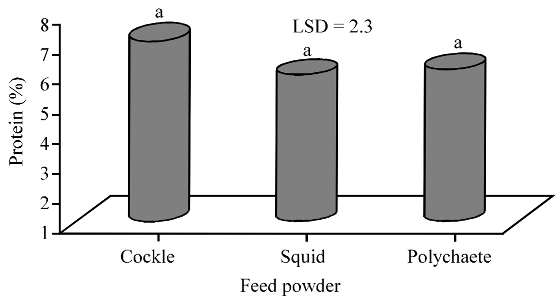

Fig. 9: Proteins percentages of offered different fresh food to the shrimps (n = 43); different letters indicate significant difference among treatments ($p<0.05$). Cockle = 7.17; Squid = 5.99; Polychaete = 6.13

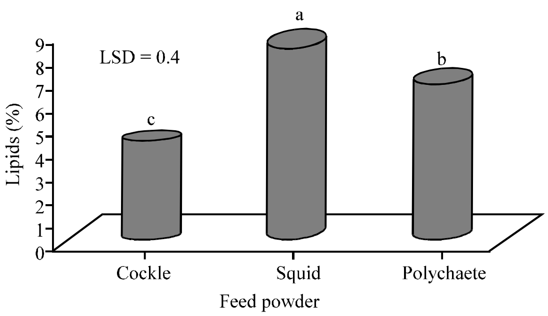

Fig. 10: Lipids percentages of offered different fresh food to the shrimps (n = 43); different letters indicate significant difference among the treatments ($p<0.05$). Cockle = 4.14; Squid = 8.52; Polychaete = 6.86

Crude protein content in offered fresh diets: Protein content of cockle diets composition was 7.2±0.1%; it was significantly higher by 20.2% than squid (5.99±1.49%). Followed by, polychaete (6.13±0.6%) which was also 2.3% higher than squid (Fig. 9).

Crude lipids content in offered fresh diets: Lipids content in squid was 8.52±0.13% and it was significantly higher by 102.8% than cockle (4.2±0.3%). Whereas, polychaete with 6.86±0.07% which was 63.3% higher than cockle (Fig. 10).

Proximate analysis of shrimps fed by fresh diets: The moisture, ash, protein and lipids content of the shrimp fed with cockle squid and Polychaete are as under:

Moisture content of shrimps fed by fresh diet shrimps: Polychaete which was fed to shrimp showed highest level of moisture (10.6±0.09%) that was significantly higher (20.5%) as compared to the control (8.8±0.08%).

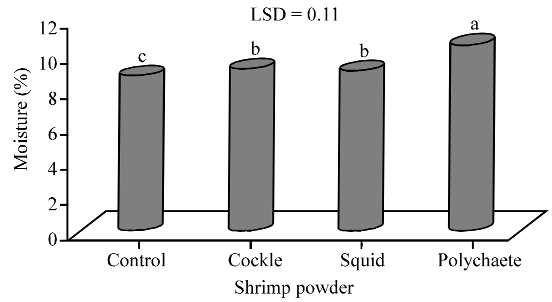

Fig. 11: Moisture percentages in shrimp fed by different fresh food (n = 43); different letters indicate significant difference among the treatments ($p<0.05$). Control = 8.86; Cockle = 9.20; Squid = 9.10; Polychaete = 10.62

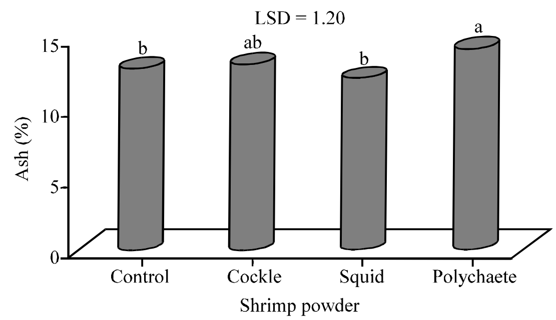

Fig. 12: Ash percentages in shrimp fed by different fresh food (n = 43); different letters indicate significant difference among treatments ($p<0.05$). Control = 12.87; Cockle = 13.19; Squid = 12.18; Polychaete = 14.27

Cockle and squid showed 9.2±0.05 and 9.1±0.06% of moisture, respectively which were also higher than the control by 4.5 and 3.4%, respectively (Fig. 11).

Ash content in shrimp fed by fresh diets: Polychaete which fed to shrimp had the highest level ash (14.3±1.12%) it was significantly higher about 11.7% as compared to the control (12.8±0.18%). Followed by cockle (13.2±0.30%) which was 3.12% higher than control. Unlike, the polychaete and cockle, squid (12.2±0.18%) was decreased in percentage about 4.9% as compared to control (Fig. 12).

Protein content in shrimp fed with fresh diets: Cockle, squid and polychaete fed to shrimp had the low level of protein which were 7.2±0.21, 6.9±0.41 and 7.2±0.08%, respectively. It was decreased in percentages about 6.9, 11.6 and 6.9% as compared to the control (Fig. 13).

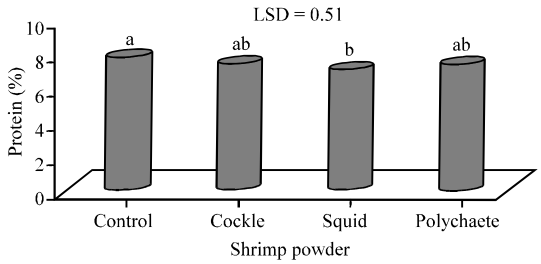

Fig. 13: Proteins percentages in shrimp fed by different fresh food (n = 43); different letters indicate significant difference among the treatments (p<0.05). Control = 7.67; Cockle = 7.20; Squid = 6.91; Polychaete = 7.18

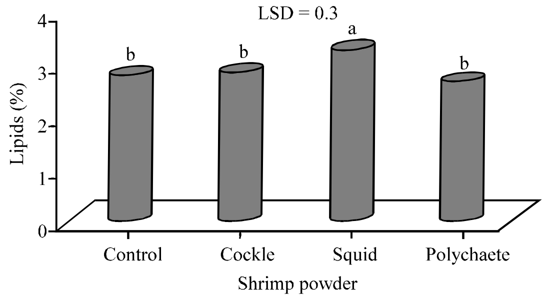

Fig. 14: Lipids percentages in shrimp fed by different fresh food (n = 43); different letters indicate significant difference among treatments (p<0.05). Control = 2.78; Cockle = 2.81; Squid = 3.27; Polychaete = 2.64

Crude lipids content in shrimp fed by fresh diets: Squid fed to shrimp had the highest level lipids (3.3±0.15%), it was significantly higher about 17.85% as compared to the control (2.8±0.15%). On the other hand cockle was recorded similar (2.8±0.08%) as control. Unlike, cockle and squid, polychaete (2.6±0.3%) was decreased in percentage about 7.7% as compared to the control (Fig. 14).

DISCUSSION

In this study, varieties of foods have been used to achieve the ideal success for gonad maturation. The difference in lipids, protein, ash and moisture was recorded among fresh food items were used in this study. The findings indicated that fresh squid treatment was necessary for the gonad maturation. Browdy (1998) and Peixoto *et al*. (2005) reported that the variety of fresh food has guaranteed the success of penaeid maturation in captivity. However, the effects of various diets in maturation system of spermatophore quality of *P. merguiensis* are currently in progress. It was well documented that organic compounds in penaeid, dietary lipids and protein were involved in gonad maturation process (Samuel *et al*., 1999; Wouters *et al*., 2002; Perez-Velazquez *et al*., 2003; Meunpol *et al*., 2005; Coman *et al*., 2007). These compounds are the most abundant in foods are used in biosynthesis and mobilized during maturation (Harrison, 1990). In present study, it is observed that spermatophore weight has no significant difference among the treatments which is well correspond with previous study by Nakayama *et al*. (2008). Leung-Trujillo and Lawrence (1987) and Alfaro (1993) have reported that the post harvesting processes 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 broodstock in captivity.

Spermatophore renewal completes in every 2 or 3 weeks (Pascual *et al*., 1998). However, in this study spermatophore of *P. merguiensis* takes 4-5 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). It was well documented that the food is an important factor for the sexual maturation of gametes and improves male reproduction performance (Browdy, 1992; Meunpol *et al*., 2005). Hence, it postulates that food might have a considerable role in the spermatophore maturation.

Inclusion of fresh food items during the maturation of *P. merguiensis* male is important to reduce the effects of natural spermatophore degeneration. The fresh food diet can be used without reproductive disruption in males as shown in previous studies with others species of penaeid (Browdy, 1992, 1998; Harrison, 1997; Peixoto *et al*., 2005). The highest percentage of normal sperm in relation to body weight and age was reported by Alfaro (1993). At the early maturation stage of spermatophore, there were very few numbers of matured sperms observed (Wang *et al*., 1995). Similar observation was also reflected in present study. Interestingly, it was recorded *P. merguiensis* produces fully matured sperms at 6th week of dietary treatment (Fig. 3).

CONCLUSION

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

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