Production of artificial diets for female broodstock of western white shrimp (*Litopenaeus vannamei*) and study on their singular effect

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Abstract

Advantages of pelleted feeds in terms of storage, transportation and lower costs compared to natural fresh feed justified the replacement of artificial feeds instead of natural ones partially on maturation performances. This study comprised nine treatments (with three replications) including control treatment: polychaete worm (*Perinereis nuntia*), Squid and calf liver (natural feed); T_1: pellet feed with 50% crude protein and 8% crude lipid+ *P. nuntia*, Squid and calf liver; T_2: pellet feed with 50% crude protein and 10% crude lipid+ *P. nuntia*, Squid and calf liver; T_3: pellet feed with 40% crude protein and 10% crude lipid+ *P. nuntia*, Squid and calf liver; T_4: pellet feed with 40% crude protein and 8% crude lipid+ *P. nuntia*, Squid and calf liver *P. nuntia*; T_5: pellet feed with 50% crude protein and 10% crude lipid; T_6: pellet feed with 50% crude protein and 8% crude lipid; T_7: pellet feed with 40% crude protein and 10% crude lipid; T_8: pellet feed with 40% crude protein and 8% crude lipid. In treatments 1, 2, 3 and 4, 50% of the natural diets were removed and pellet foods used instead. The amount of pellet feeds that given daily, was 3.5% of shrimp broodstock biomass. In treatments 5, 6, 7 and 8, the amount of pellet feeds that given daily was 7.5% daily. Feeding was done 4 times a day. Gonadosomatic index (%GSI) in the control (3.23%), treatment 3 (3.20%) and treatment 6 (3.02%) were significantly higher than that in the other treatments (p<0.05). Absolute fecundity in the control (29980 eggs) and treatment 3 (29683 eggs) was significantly higher than other treatments (p<0.05). Hepatosomatic index (%HSI) reached the lowest level in treatment 8 (2.14%). But in treatments 3 and 5 was more than control, but did not any statistical significant (p>0.05). Survival rate in the treatment 8 (26.66%) was significantly lower than other treatments (p<0.05). Generally, In the treatment 3, with the elimination of 50% of the natural foods, and the use of pellet food instead of them, result of comparative indices of reproduction were in desirable proportions.

Keywords: Natural wet feed, Pellet feed, Sexual maturation, Western white shrimp female brood

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Introduction

It has been reported that total replacement of natural feeds with artificial ones has not been achieved, yet (FAO, 2010). Several factors have made it impossible to use large amount of pellet feed for shrimp broodstock' maturation. Chimsung (2014) have reported that polychaete worm improves shrimp reproduction due to having high nutritional value, arachidonic acid and important reproduction hormones. In addition, it has been believed that polychaete worm is an excellent source for not only unsaturated fatty acids, but also sexual hormones similar to those of shrimp. Also, squid not only is a rich source of cholesterol, but also contains sexual steroids involved in vitellogenesis (Wouters *et al.,* 2005). As mentioned before, natural feeds for breeder feeding have high amounts of n-3 fatty acid. Also, fecundity rate of broodstock is correlated with n-3 fatty acid (Wouters *et al.*, 2005).

Amino acids, fatty acids, protein and lipid play an important role in shrimp breeder maturation and need more protein during the sexual maturation period (Coutteau, 2001). In addition, highly unsaturated fatty acids (HUFA) especially eicosapentaenoic acid and docosapentaenoic acid are important components of breeder diets and are found abundantly in ovary tissue (Teikwa and Mgaya, 2003). Diets deficient in n-3 HUFA adversely affect ovary development, fecundity and egg quality (Teikwa and Mgaya, 2003). Western white shrimp broodstock with frequent spawning have higher protein content in their hepatopancreas and ovaries than those broodstock with weak spawning (Magalhaes *et al*., 2012). Successful sexual maturation in western white shrimp can be obtained with freshly-frozen wet diet (with or without artificial diets). The researchers have emphasized that the weight of matured shrimp ovaries may increase 4-6 fold within about one week (Verstraete *et al.,* 1995). Divan *et al*. (2009) reported that pelleted feeds containing 50% protein and 10% lipid can be used in combination with fresh feeds. Alava *et al*. (1993) reported that diets lacking HUFA cause a decrease in gonado-somatic index (GSI). Consequently, it is believed that HUFAs are needed for sexual maturation. Pelleted feeds are important in many aspects, like ease of storage, transportation, feeding and low costs. The aim of the present study was to provide a suitable pelleted diet for the sexuall maturation of female broodstock and determining their effects on sexuall parameters.

Materials and methods

Broodstock acclimation

A total number of 270 female broodstock with an average weight of 37±2 g were sampled from Bandargah Research Station and kept in two 4-ton fiberglass tanks for one week to acclimatize prior to stocking in 300-L tanks. Eyestalk ablation was done a week after stocking in the 300-L tanks

filled with 150 L water. Feeding and sexual maturation in broodstock were checked every day for 5 weeks.

Treatments

This study was conducted with 9 treatments each with three replications containing 10 broodstock. Treatments included control: polychaete worm (*P. nuntia*), Squid and calf liver (natural feed); T_1: pellet feed with 50% crude protein and 8% crude lipid+ *P. nuntia*, Squid and calf liver; T_2: pellet feed with 50% crude protein and 10% crude lipid+ *P. nuntia*, Squid and calf liver; T_3: pellet feed with 40% crude protein and 10% crude lipid+ *P. nuntia*, Squid and calf liver; T_4: pellet feed with 40% crude protein and 8% crude lipid+ *P. nuntia*, Squid and calf liver *P. nuntia*; T_5: pellet feed with 50% crude protein and 10% crude lipid; T_6: pellet feed with 50% crude protein and 8% crude lipid; T_7: pellet feed with 40% crude protein and 10% crude lipid; T_8: pellet feed with 40% crude protein and 8% crude lipid.

Feeding rate (natural feeds) was 25% of shrimp broodstock biomass daily (Brock and Main, 1994). Their composition is detailed (Tables 1 and 2). In treatments 1, 2, 3 and 4, 50% of the natural diets were removed and pellet foods used instead. The amount of pellet feeds that given daily, was 3.5% of shrimp broodstock biomass. In treatments 5, 6, 7 and 8, the amount of pellet feeds that given daily was 7.5% daily. Feeding was done 4 times a day.

Feed types

Wet feed

Polychaete worm *(P. nuntia)*, Squid and calf liver were used for the broodstock feeding and sexual maturation.

Pellet feed

Pellet feeds were manufactured with laboratory equipment. In addition, depending to treatment type, four pellet feeds were prepared.

Analyses

Shrimp length including carapace length and total length were measured by a digital calipers (Model TITAN), whereas, total wet weight was measured using a digital scale (Model WANT) at 0.001 g nearest (Conides *et al*., 2008). The sexual stage, and weight of ovaries, oocyte count, fecundity and hepatopancreas weight in female broodstock were determined (Islam *et al*., 2012). Also, eyestalk ablation and sexual development assessment of broodstock was performed according to Aelgamal (2015). Water temperature, dissolved oxygen, salinity and pH were measured daily. Data were analyzed using one-way ANOVA and the differences among the treatments were determined using Duncan test (α=0.05). All statistical analyses were performed in SPSS 17.

Table 1: Proximate composition of the pelleted feeds for the brood shrimps feeding.

Food	Composition (%)				
	Crude protein	Crude fat	Ash	Fiber	Moisture
Pellet 1	50	10	6.65	4.32	7.4
Pellet 2	50	8	6.35	4.12	7.52
Pellet 3	40	10	5.48	3.32	7.32
Pellet 4	40	8	5.38	3.19	7.88

Table 2: Proximate composition of the natural feeds for the brood shrimps feeding.

Food	Composition (%)				
	Crude protein	Crude fat	Ash	Fiber	Moisture
Perinereis nuntia	5.85	3.63	10.45	0.79	12.41
Squid	17.02	7.92	19.57	0.11	20.66
Calf liver	19.6	3.95	13	-	2.3

Results

Proximate composition of the feed ingredients is shown in Table 3.

The first sexual maturation was observed in treatments 2 and 7. Number of the broodstock in different sexual maturation stages during the experiment is presented in Table 4.

Table 3: Proximate composition of the feed ingredients.

Feed ingredients	Composition (%)				
	Crude protein	Crude fat	Ash	Fiber	Moisture
Shrimp meal	51.5	5	6	2	19.34
Wheat meal	11.74	1	1	8	10.48
Soybean meal	46.4	2	6	2.6	7.65
Fish meal	71.31	9	9	1	5.59
Squid meal	49.17	6	9	3	4.52

Table 4: Mean number of the brood shrimps with different maturation stages during the experiment.

Items	Control			1			2			3			4			5			6			7			8		
	Maturity stage			Maturity stage			Maturity stage			Maturity stage			Maturity stage			Maturity stage			Maturity stage			Maturity stage			Maturity stage		
Number	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
	10	12	6	12	13	6	16	10	4	14	7	6	16	9	4	15	14	1	10	19	9	13	6	1	7	2	0
Average	3.05±9.33[a]			3.78± 10.33[a]			6± 10[a]			4.35±[a] 9			6.02 ±9.66[a]			7.81± 10[a]			5.5± 12.66[a]			6.02±6.66[b]			1.24±3.12[c]		

Different letters show significant difference among the treatments (p<0.05). In the treatments control, 1, 2, 3 and 4 were observed, respectively 2, 1, 1, 3 and 1 brood shrimp in stage 4 of maturation.

Growth and reproduction indices are presented in Table 5 and Figs. 1-5. The growth indices of ovaries in the control, treatment 3 and treatment 6 were significantly higher than that in the other treatments. HSI was similar in most treatments and showed some significant difference. Absolute

fecundity of the control and treatment 3 was significantly higher (p<0.05) than that in the other treatments. Survival rate in treatment 8 was significantly lower (p<0.05) than that in the other treatments.

Table 5: Mean (±SD) of growth and reproductive indices in different treatments.

Features	Control	1	2	3	4	5	6	7	8
Weight (g)	4.77±39.06^{a}	6.17±39.72^{a}	3.34±39.14^{a}	3.43±39.68^{a}	3.45±38.34^{a}	3.47±36.82^{a}	2.43±37.61^{a}	1.52±36.23^{a}	2.83±37.6^{a}
Carapace length (cm)	0.13±2.54^{a}	0.21±2.56^{a}	0.16±2.57^{a}	0.12±2.58^{a}	0.08±2.57^{a}	0.12±2.55^{a}	0.11±2.52^{a}	0.11±2.52^{a}	0.05±2.48^{a}
Body length(cm)	0.78±15.47^{ab}	1.19±15.33^{ab}	0.64±15.58^{ab}	1.08±16.05^{a}	0.5±15.66^{ab}	0.79±14.96^{b}	0.77±14.82^{b}	0.54±15.17^{ab}	0.25±15.16^{ab}

In each row, different letters show significant difference among the treatments (p<0.05).

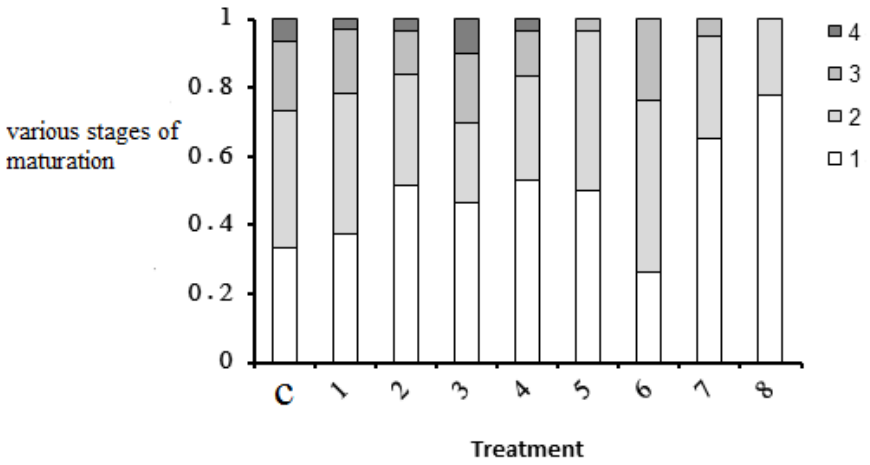

Figure 1: Correlation between the treatments and frequency of various stages of maturation.

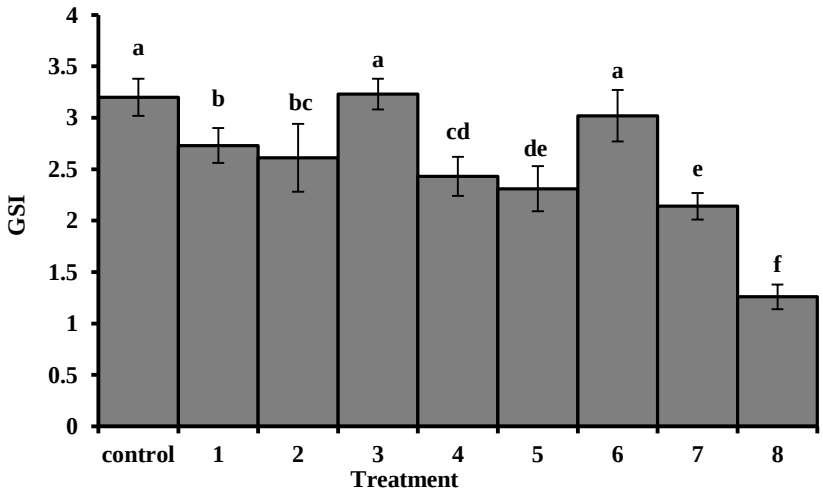

Figure 2: Effect of different treatments on GSI (different letters above the bars mean significant difference at α=0.05).

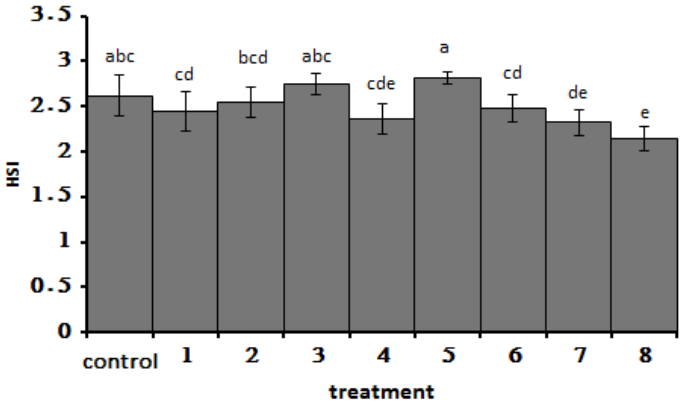

Figure 3: Effect of different treatments on HSI (different letters above the bars mean significant difference at α=0.05).

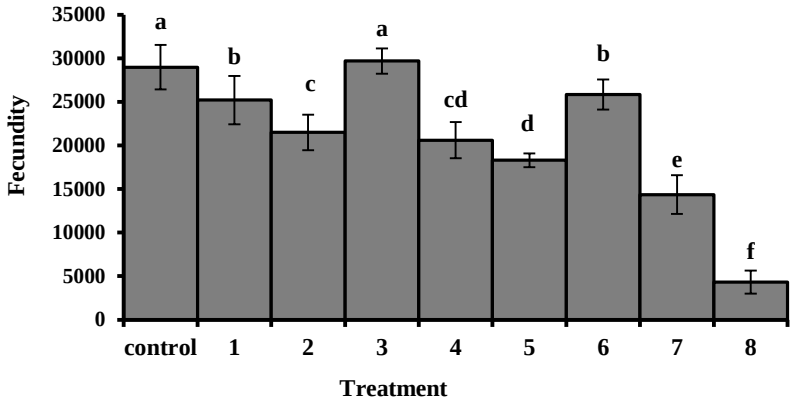

Figure 4: Effect of different treatments on fecundity (different letters above the bars mean significant difference at α = 0.05).

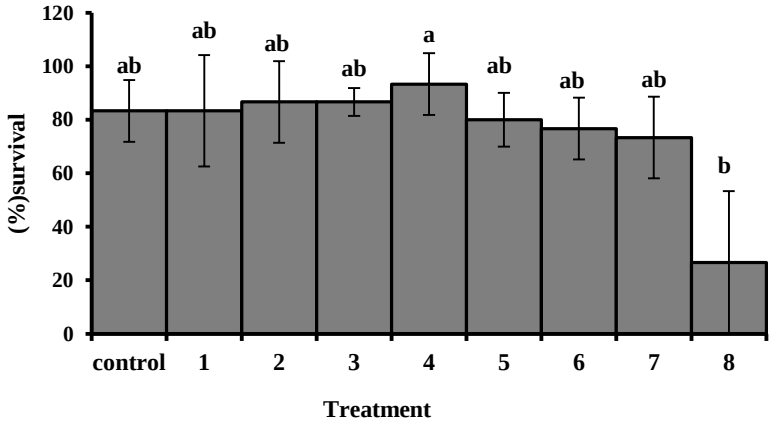

Figure 5: Effect of different treatments on survival rate (different letters above the bars mean significant difference at α = 0.05).

There were no significant differences (p>0.05) in survival rate and final weight of broodstock among the treatments. The broodstock length in the treatments 5 and 6 was significantly lower than that in the other treatments (Table 5).

Mean (±SD) of water physico-chemical parameters during the experiment are presented in Table 6.

Table 6: Mean (± SD) of water physico-chemical parameters during the experiment.

Parameters	Water temperature (^{o}C)	Dissolved oxygen (mg/L^{-1})	Salinity (ppt)	pH
amount	30±1	6.93±0.53	33±2	8.16±0.11

Discussion

At present, the nutritional requirements of brood shrimps are hard to supply in propagation centers. The main problems with fresh wet feeds (worm and squid) are their availability, high price, difficulty of transportation and washing prior to use. Thus, in this study 50% and 100% of natural wet feeds were replaced with pellet feeds to investigate the possibility of the replacement of pelleted feeds to wet feeds. Also, it has been reported that total replacement of natural feeds with artificial ones has not been achieved, yet (FAO, 2010). Several factors have made it impossible to use large amounts of pellet feeds for shrimp breeder maturation. Chimsung (2014) reported that polychaete worm, improves shrimp reproduction due to having high nutritional value, arachidonic acid and important reproduction hormones. In addition, it has been believed that polychaete worm is an excellent source of not only unsaturated fatty acids, but also sexual hormones similar to those of shrimp. Also, it was reported that squids contain high levels of protein, HUFA and cholesterol, which are necessary for successful growth and reproduction of shrimp (Chimsung, 2014). In addition, it has been reported that bloodworm, squid, mussel and clam meat along with pelleted feeds should be used for shrimp feeding (Brock and Main, 1994). Divan *et al*. (2009) reported that pelleted feeds containing 50% protein and 10% lipid (PUFA) can be used in combination with fresh feeds. However, the rate of replacement of fresh feeds with pelleted feeds has not been suggested. Thus, in the present study, the effect of even 100% replacement was investigated on brood shrimp. Sangpradub *et al*., (1994) investigated the effect of different feeding regimes on ovary maturation and spawning of black tiger shrimp (*Penaeus monodon*) and in line with the results of present study, found that the highest ovary maturation and spawning was related to the broodstock fed either wet feeds or wet feeds plus pelleted feeds.

As, mating occurs predominantly when female broodstock are in stage 4 of sexual maturation, low mating frequency means a few of the broodstock are in stage 4. Such a condition was observed in the present study considering that there were 10 broodstock in each 300-L tank. In the investigation of the breeder sexual maturation, stress delivered by tank siphoning and water replacement should be considered, particularly in small tanks.

In the case of the importance of dietary protein in shrimp broodstock reproduction, Wouters *et al*. (2001) reported that due to synthesis of new biotic materials during the sexual maturation, the animals need higher protein. Also, they reported that protein content of artificial feeds is about 50% depending on shrimp species and protein source. Wouters *et al*. (2004) found an increase in ovary protein content during oocyte development and reproduction. Requirement for dietary lipid depends on HUFA, phospholipids, sterols and energy supplementation. For a long time it has been known that the crustaceans have little ability to synthesis HUFA, phospholipids and sterols (Sangpradub *et al*., 1994). Artificial feeds have seemingly lower EPA (20:5n-3) compared to natural eeds, thus, they have lower n-3-HUFA and DHA: EPA ratio. Diets for the shrimp brooders during sexual maturation should have high n-3/n-6 ratios. It seems that, they require dietary phospholipids (Sangpradub *et al*., 1994). Verstracte *et al*. (1995) reported that successful sexual maturation of western white shrimp can be met using freshly-frozen marine organisms without formulated diets. The results of present study showed that, it is possible to eliminate 50% of natural diets and use pellet feed instead.

ACE (2003) reported that for western white shrimp propagation, water salinity, temperature and pH should be 32 ppt, 27-29°C and about 8 respectively and saturated dissolved oxygen of above 5 ppm is required.

Stickney (2000) recommended the following water properties for sexual maturation of tropical shrimp broodstock: salinity=27-36±0.5 ppt, temperature=27-29±0.2^{o}C, pH=7.8±0.2, and dissolved oxygen 5±0.5 mg/L^{-1}. Regarding the mentioned cases, in the present study the physic-chemical factors of water were in the right range.

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