



*Review Article*

## Maturation diets for black tiger shrimp (*Penaeus monodon*) broodstock: a review

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### Abstract

Maturation diets for shrimp ideally comprise excellent digestible protein, essential fatty acids, and cholesterol and chemo-attractant properties which are found in natural food sources in the shrimp habitat. Fresh feeds typically including polychaete worms, mollusks and crustaceans have been extensively used for shrimp broodstock. However, their nutritional value can vary with species, season of harvest and life stage. Alternatively, compound diets have been formulated based on the nutritional profile of fresh feeds have being produced in a semi-moist diet and dried pellets. But, substitution of fresh feeds by compound diets is scarce due to lack of information on the quantitative dietary requirements of shrimp broodstock. For most *Penaeus monodon* hatcheries, good reproductive performance was obtained when shrimp broodstock were fed a mixture of fresh feeds and compound feeds.

**Keywords:** maturation diet, *Penaeus monodon*, black tiger shrimp, shrimp broodstock

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### 1. Introduction

Aquaculture of black tiger shrimp, *Penaeus monodon* is an economically important industry in Thailand, however, over the past 10 years, production has been in decline due to disease outbreaks. Regardless, consumer demand for farmed black tiger shrimp remains high. The use of domesticated broodstock as opposed to captive wild broodstock may help the industry produce pathogen-free larvae and represents one of the most important strategies to successful shrimp farming activities. Several factors have a high impact on egg quality and quantity such as broodstock age and/or size, broodstock origin, type of endocrine manipulation, genetic variation and broodstock nutrition (Racotta *et al.*, 2003). An adequately formulated maturation diet which meets the nutrient requirements of shrimp broodstock is the one of the

most important criteria. Many investigations on various nutritional factors that play critical roles in the stimulation of shrimp sexual maturation, enhancement of fertility and the production of viable, high-quality offspring have been reported. Several studies have indicated that fresh feeds (e.g. clam, squid, polychaete worms, *Artemia*) can promote successful reproductive performance of shrimp broodstock. However, fresh feeds present several disadvantages such as the increased risk of disease transmission, variable nutritional quality, unpredictable supply and the potential to cause deterioration of water quality in hatchery culture systems (Harrison, 1990; 1997). The use of compound diets is advantageous because they are easier to manage and store and, in addition, they present less danger of pathogenic contamination (Wouters *et al.*, 2000). However, the lack of clearly defined nutritional requirements for black tiger shrimp broodstock presents a knowledge gap for adequate diet formulation and, thus far, results have been unsatisfactory when compared with fresh feeds (Wouters *et al.*, 2002; Meunpol *et al.*, 2005). The objective of the present review is to summarize the current state of knowledge on the develop-

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ment of compound maturation diets for black tiger shrimp broodstock.

## 2. Fresh Feeds

The predominant feeding practice in shrimp hatcheries and in shrimp broodstock feeding trials has been used fresh feeds such as annelid worms (polychaetes), squid, bivalves (mussels, clams and oysters), crustaceans (shrimp, crab, *Artemia*) and fish.

### 2.1 Polychaetes

Feeding of polychaetes can enhance reproductive performance of shrimp due to their high nutritional composition, high unsaturated fatty acid content (e.g. arachidonic acid) and the presence of reproductive hormones (Meupol *et al.*, 2005). The main polychaete species intensively used in marine shrimp broodstock maturation diets are sandworms (*Perinereis* sp.) (Meupol *et al.*, 2005) and mudworms (*Marphysa* sp.) (Coman *et al.*, 2007).

The quantity and nutritional quality of polychaetes fluctuates from season-to-season and between geographical locations. In order to reduce the impact of over-harvesting and to produce pathogen-free polychaetes for shrimp hatcheries, a semi-sterile technique for commercial polychaete farming was developed (Poltana *et al.*, 2007). Techprempreecha *et al.* (2011) determined the nutritional composition among cultured and wild sandworms. Wild sandworms (average weight 2.73 g) were obtained from Bangphra beach, Chonburi Province, Thailand, while the cultured sandworms were supplied from a commercial sandworm farm in Samutsongkram Province (average weight 0.73g). Farmed sandworms were reared on a commercial shrimp diet. The two groups of sandworms were starved for 2 days before sampling for biochemical analysis. The freeze-dried samples were analyzed in triplicate for nutritional composition. The comparison of nutritional content (dry weight basis) in terms of protein (51.2 vs. 52.8%) and energy (219 vs. 197 kJ/g) were not different. However, fat (34.0 vs. 27.4%) and ash levels (9.4 vs. 6.7%) of cultured sandworms were significantly higher than those of wild sandworms. Fatty acid composition in

term of arachidonic acid (3.3 vs. 3.1% of total fatty acid) and docosahexaenoic acid (1.5 vs. 1.1% of total fatty acid) were similar between cultured and wild sandworms, but ecosapentaenoic acid (4.2 vs. 3.0% of total fatty acid) was higher in wild sandworms. Total PUFA content was higher in cultured sandworms (24.7% of total fatty acid) than in wild sandworms (18.4% of total fatty acid). The overall nutritional value indicated that cultured sandworms reared on a commercial shrimp feed are a suitable diet for shrimp broodstock.

Polychaetes are not only an excellent source of HUFA but possibly also a good source of reproductive hormones, similar to those found in shrimp. To date, reproductive hormones of polychaetes such as progesterone (P4) and 17 $\alpha$ -hydroxyprogesterone have been reported to be capable of inducing shrimp oocyte development (Meupol *et al.*, 2007). In addition, prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) extracted from polychaetes had a positive effect on oocyte maturation especially during the late maturation and ovulation period (Meupol *et al.*, 2010).

### 2.2 Mollusks

Mussels, clams and oysters are commonly fed to shrimp broodstock either fresh live or as a previously frozen meat. The nutritional content of mollusks varies considerably (Table 1). Mollusk meat is a good source of essential nutrients for shrimp and, in addition, possesses excellent chemo-attractant properties (Hertrampf and Piedad-Pascual, 2000).

### 2.3 Squid

Squid mantle, head and tentacles are often used for human consumption. After processing, the squid by-product (e.g. viscera, fins, skin and pen) typically amounts to about 52% of the whole weight. These by-products are then made into a meal which can be used as a high-quality feed ingredient for fish, shrimp and other animals (Hertrampf and Piedad-Pascual, 2000). When the whole squid is used for feeding shrimp broodstock, fresh or frozen squid are usually chopped before being fed to shrimp broodstock.

Table 1. Chemical composition of bivalve meat (% dry matter)

	Blue mussel	Green mussel	Brown mussel	Clam	Oyster
Moisture	-	-	85.6	-	83.1
Protein	60.4	52.5	62.5	56.5	48.0
Lipid	8.5	15.4	7.9	8.8	10.8
Fiber	-	3.1	11.6	-	3.8
Ash	11.6	9.3	5.1	9.6	10.8
N-free extract	19.5	19.7	12.7	11.2	24.6

Adapted from Hertrampf and Piedad-Pascual (2000)

The summarized data presented in Table 2 indicate that squid has the highest level of protein but contains low lipid levels; however, the portion of HUFA of the total lipid is high (Hertrampf and Piedad-Pascual, 2000). Squid meat is an excellent source of cholesterol which is an essential dietary nutrient for shrimp growth and successful reproductive performance. Of the total lipid present in squid mantle, 18.0% is cholesterol, which is higher than any other animal feed ingredient (Akiyama, 1992).

The most commonly used fresh feeds for *P.monodon* broodstock are highly variable in their nutritional composition. The data presented in Table 2 indicates that squid typically have the highest protein content (>80%), followed by polychaetes and oysters (>50%). Lipid contents are highest in farmed and wild sand polychaetes (>14%), followed by oysters (>12%), while squid and farmed mud polychaetes contain relatively low lipid levels (<6%). The percentage of HUFA contained in polychaetes, squid and oysters is shown in Table 3. Arachadonic acid (AA) is high (>7%) in polychaetes while squid is the richest (>12%) in docosahexaenoic acid (DHA).

### 3. Feeding Practices Using Fresh Feeds

Fresh feeds are usually fed to shrimp at a ratio of 10-15% of shrimp body weight four times daily; however, this can vary widely between different hatcheries and laboratories. Some laboratories wash and soak fresh feeds in fresh-water for 30 min to eliminate marine parasites before feeding to shrimp (Hoa *et al.*, 2009). Supplementation of sodium alginate (Chung *et al.*, 2011) and *Spirulina* (Wiriyapattanasub *et al.*, 2011) into fresh feeds can promote a healthy shrimp reproductive system. There are two shrimp broodstock feeding practices typically used in Thailand: Single fresh feeds or composite diets.

#### 3.1 Single fresh feed

Shailender *et al.* (2012) determined the significance of different fresh diets to male reproduction. They compared three different fresh feeds; fresh squid, oyster and polychaete on sexually matured male shrimp collected from the Indian coast. A total of 50 males for each treatment with mean body weight of 61.58-69.32 g were used. For wild caught shrimp reared under controlled conditions, the feeding period was

up to 5 weeks with the observation of reproductive performance of shrimp by spermatophore weight, sperm count and viability. The results in Table 4 indicated that fresh squid had superior results for reproductive performance of male shrimp.

Serious viral disease like white spot syndrome virus (WSSV), yellow head virus (YHV) and Taura syndrome virus (TSV) caused high mortality and serious damage to the shrimp culture industry. In an effort to mitigate crop losses from those disease problem, some approaches have been developed such as selective breeding of specific pathogen-free (SPF) and specific pathogen-resistant (SPR) shrimp and then culturing them in a biosecure system (Lotz, 1997). In order to resolve disease problem, feeding strategies based on supplementation of immunostimulants in shrimp farms have been studied (Chung *et al.*, 2011). Alginic acid, a polysaccharide component isolated from the cell wall of brown algae, has been used as an immunostimulant in diet of maturation by enriching polychaete with sodium alginate (Chung *et al.*, 2011). Incorporating sodium alginate into polychaete sea worm was carried out by a sodium alginate injection. Briefly, the fresh saline solution of sodium alginate at a concentration of 200mg/kg was injected into the coelom of live polychaete. The sodium alginate injected sea worms were immediately fed to tiger shrimp broodstock. During the feeding trial, each female and male continued to be fed experimental polychaete at a feeding rate around 20-25% of shrimp body weight, four times daily at 0600, 1100, 1700 and 2200. Reproductive performance of female, and larval quality were observed. Results indicated that sodium alginate treatment had no effect on mortality of shrimp broodstock or

Table 2. Total protein and lipid (% dry weight) of fresh feeds used to feed *P. monodon* broodstock

Fresh feed	Protein	Lipid
Sand Polychaetes(wild) <sup>1</sup>	52.8	27.4
Sand Polychaetes (farmed) <sup>1</sup>	51.2	34.0
Sand Polychaetes (farmed) <sup>2</sup>	63.9	14.2
Mud polychaetes (farmed) <sup>2</sup>	50.9	5.3
Squid <sup>2</sup>	84.5	3.1
Oysters <sup>2</sup>	51.7	12.2

<sup>1</sup>Techaprempreecha *et al.* (2011); <sup>2</sup>Meunpol *et al.* (2005)

Table 3. HUFA content (%) of fresh feeds used to feed *P. monodon* broodstock

HUFA	Polychaetes	Squid	Oysters
Arachidonic Acid (AA)	7.65	5.35	0.75
Eicosapentaenoic Acid (EPA)	7.54	9.12	1.48
Docosahexaenoic Acid (DHA)	2.35	12.39	0.75

Shailender *et al.* (2012)

Table 4. Performance of male shrimp fed different fresh feeds

	Polychaete	Squid	Oyster
% survival	100	100	100
Initial weight (g)	65.24±1.6	69.32±1.5	61.58±2.5
Final weight (g)	75.41±3.6	78.65±3.8	68.25±1.9
Sperm sac weight (g)	0.1062	0.1221	0.09762
Total sperm (x10 <sup>6</sup> )	35.69	39.47	32.48
% of live sperm	82.64	92.57	76.25
% of abnormal sperm	32.16	28.74	26.24

Adapted from Shailender *et al.* (2012)

spawning frequency. However, sodium alginate enriched sea worms led to improved tiger shrimp broodstock reproductive performance, including increasing broodstock fecundity and larval production.

### 3.2 Composite diet

A diversified fresh feed mixture approach provides a better balance of dietary nutrient. A combination of natural feed yielded better reproduction than diet of single natural feed has been proved in *P. vannamei* under the investigation of Chamberlain and Lawrence (1981). Shrimp broodstock were fed single-feed diets (clams, shrimp, squid, and worms) and one composite diet consisting of all four. Ovarian maturation, spawning, molting rate, and survival were monitored daily in addition, growth and gonad size were measured at termination. The composite diet was the best overall diet, while squid was the best single-feed, followed by shrimp, worms, and clams.

Research conducted in Vietnam on the effect of composite diet on *P. monodon* reproductive performance exhibited a positive effect. Proportion of fresh feed ingredients namely oyster, pork liver, squid and marine worm at the portion 29:11:42:18 % of wet weight, respectively formulated based on the fatty acid profile of mature ovaries in term of AA/EPA and DHA/EPA ratio, enhanced growth and fecundity of broodstock shrimp (Hoa *et al.*, 2009).

A longer period of captive shrimp rearing under feeding of fresh feed often does not provide adequate levels of nutrients needed by broodstock shrimp and it increases the risk of disease transmission and offspring quality. The nutritional quality of fresh feed can be effectively improved by enrichment technique. A study in pink shrimp (*Farfantepenaeus paulensis*) fed fresh feed supplemented with pollen showed higher sperm counts and increase larval quality (Braga *et al.*, 2013). Several research works have demonstrated the positive effects of including carotenoids in broodstock diet affecting growth and reproductive performance (Wouters *et al.*, 2001), and improving embryonic and larval development (Regunathan and Wesley, 2006).

Carotenoids play an important role because shrimp are unable to synthesize carotenoid *de novo* and depend entirely on carotenoids in their feed (Yamada *et al.*, 1990; Harrison, 1997). Carotenoids have been suggested to have the capacity to trigger shrimp vitellogenesis and the effect is directly related to the transcription of hormone genes involved in maturation of the ovary (Linan-Cabello *et al.*, 2004).

More recently, feeding practices and maturation diet strategies have increased the amount of carotenoid in natural food of *P. monodon* by enrichment with *Spirulina*, which is a rich source of carotenoid, into natural food before feeding to female shrimp brood stock. Wiriapattanasub *et al.* (2011) used *Spirulina* enriched blood clam (*Anadara granosa*) as

Table 5. Feeding program for *P. monodon* broodstock

Treatment	Feeding time		
	0500h	1300h	2200h
Treatment A	Swimming crab (20% shrimp body)	Sand worm (10% shrimp body)	Squid (20% shrimp body)
Treatment B	Swimming crab (20% shrimp body)	<i>Spirulina</i> -enriched blood clam (10% shrimp body)	Squid (20% shrimp body)

Wiriapattanasub *et al.* (2011)

a maturation diet for female *P.monodon* broodstock from the Andaman sea offshore of Satun province, Southern of Thailand. They fed blood clam with *Spirulina* (spray dried powder, food grade, ASB SPIRUMATE, Advanced *Spirulina* Biotechnology Co.,Ltd, Thailand) at 30% of body weight (30g *Spirulina* for 100 g of blood clam) for 3 days before feeding it to broodstock. Different portions of fresh feed (Table 5) were fed to shrimp for the duration of 30 days. The results indicated no significant difference of reproductive performance in terms of number of spawning and egg hatching rate among female broodstock fed different diets. Total carotenoids in eggs of shrimp fed *Spirulina*-enriched blood clam was twice as high as in the other group. It was clearly evident that blood clam enriched with *Spirulina* could be used as maturation diet. In addition, it is possible to use them as an alternative to sand worm, which is a high nutritional food but costly.

#### 4. Compound Diet

Although fresh feeds are used in maturation diets for penaeid shrimp, their nutritional value can vary with species, season of collection and life stage (Bray and Lawrence, 1992). Furthermore, their use is labor intensive and significantly affects water quality (Perez-Velazquez *et al.*, 2002). Partial or total replacement of fresh feed with compound diet in shrimp broodstock has been investigated. However, due to lack of information on the quantitative dietary requirements of shrimp broodstock most compound diets are formulated based on

fresh feed nutritional profile such as fatty acid profile of polychaete (Meunpol *et al.*, 2005). Only a few studies (Millamena, 1989; Quinitio *et al.*, 1996; Marsden *et al.*, 1997; Meunpol *et al.*, 2005; Paibulkichakul *et al.*, 2008) have been performed to incorporate compound diet in a moist feed or dry formulated feeds in maturation diets of *P. monodon*.

#### 4.1 Semi-moist diet

Successful alternation of fresh feed with compound diets have been reported. Marsden *et al.* (1997) assessed the effect of two soft pellet diets (BIARC1 vs. BIARC2) on female *P. monodon* reproductive performance. The pellets were designed to completely replace the fresh-frozen feed. The formulation of compound diets is shown in Table 6 using squid and mussel meal as the main ingredient. Replacing fish roe with calf liver, *Artemia* enrichment and cod liver oil in test diet was investigated. Moisture content ranged from 74.0-78.5%. The protein and lipid content in diet was in the range 52.2-54.6% and 10.7-15.6%, respectively. The reproductive performance measured over a 42 days period indicated that female shrimp fed semi-moist diet had a better larvae quality and higher spawning than shrimp fed the squid-mussel control diet. However, spawner survival, fecundity and hatch rate were not affected by the test diets.

A semi-moist diet based on fatty acid profile of polychaete on maturation of male *P. monodon* was formulated by Meunpol *et al.* (2005). The semi-moist diet contained HUFA with a ratio of 5:1:1 (AA:EPA:DHA) with the composition of

Table 6. Composition of the experimental moist diets

Ingredients	Weight (g/100g dry diet)	
	BIARC1	BIARC2
Squid ( <i>Loligo</i> sp.) meal	41.0	41.0
Minced mussel ( <i>Perna canaliculatus</i> )	23.0	22.0
Fish roe ( <i>Hoplostethus atlanticus</i> )	12.0	0
Calf liver	0	11.0
Artemia enrichment (dry Selco)	0	4.0
Cod liver oil	2.4	0
Binder mix	10.0	10.0
Milled mollusk shell ( <i>Cystiscus deltooides</i> )	2.0	2.0
Lecithin	3.0	3.0
Vitamin mix	5.0	5.0
Mineral mix	3.0	3.0
Cholesterol	1.1	1.1
Astaxanthin	4.0x10 <sup>-3</sup>	4.0x10 <sup>-3</sup>
β-carotene	4.0x10 <sup>-3</sup>	4.0x10 <sup>-3</sup>
Nutritional composition		
Crude protein	52.2	54.6
Lipid	15.6	10.7
n-3/n-6	5.5	4.5

Marsden *et al.* (1997)

Table 7. Feed ingredients of broodstock shrimp diet

Ingredients	Dry weight (g/100 g of diet)
Defatted fish meal	41
Squid meal	12
Soybean meal	8
Shrimp head meal	8
Wheat	8
Sodium alginate/Calcium alginate	4/2
Cholesterol/Lecithin	0.5/0.5
Vitamin mix/Mineral mix	2/2
Arachidonic oil	7
EPA oil	3
Fish soluble	1
Vitamin C	0.5
Astaxanthin	0.5

Meunpol *et al.* (2005)

feed ingredients presented in Table 7. The moisture, protein and fat content of semi-moist diets were 30.5, 46.8 and 14.1% dry matter, respectively. The feeding trial was performed by feeding semi-moist diet plus fresh feeds (squid, mussels, polychaete at ratio 1:1:1) at 1:1 to wild and farmed male shrimp for a month. The results exhibited comparable reproductive characteristics of male shrimp fed semi-moist diet with natural foods and the diet had a greater effect on enhancing shrimp reproduction than commercial shrimp pellet.

Semi-moist diets exhibit a moderate effect on shrimp reproductive performance but generally they have a shorter shelf life and lower water stability than dry diets. Those properties of moist diet may make them less practical in commercial hatcheries. The advantage of a feeding strategy with modified dry formulated diet with a specific nutrient composition, are costless, facilitates storage and feeding and

has lower impact on water quality (Perez-Velazquez *et al.*, 2002).

#### 4.2 Dry-compound diet

The early study on dried-compound diet formulation used squid meal as the main protein source. Table 8 presents the ingredients composition of original version by Millamena (1989) and modified formula by Qunitio *et al.* (1996). The response of pond-reared *P. monodon* broodstock to Millamena (1989) diet which was whole feeding, produced lower spawn quality compared to the modified diet from Qunitio *et al.* (1996), which was a combination feeding with natural fresh feeds. Qunitio *et al.* (1996) suggested that the performance was possibly related to the concentration of carotenoids and other components like essential fatty acids

Table 8. Feed ingredients of *P. monodon* broodstock diet (dry weight, g/100 g of diet)

Ingredients	Millamena (1989)	Qunitio <i>et al.</i> (1996)
Squid meal	30	30
Shrimp head meal	20	20
Fish meal	20	20
Wheat flour	5.5	10
Seaweed	4	4
Rice bran	5.2	-
Mineral mixture	6	2
Vitamin mixture	2.7	3
Dicalcium phosphate	-	2
fish oil	6	6
Lecithin	-	2
Cholesterol	-	1
Buthylated hydroxyl toluene	0.1	-

from natural fresh feed.

It is well known that lipids and carotenoids, specifically astaxanthin, are the important nutrients affecting reproductive performance of shrimp. This fact has been proven by Paibulkichakul *et al.* (2008). They reported the positive effect of dietary fish oil (HUFA), in particular 20:4n-6 and 22:6n-3, in addition to astaxanthin supplementation. They suggested broodstock shrimp diets supplemented with 300 mg/kg astaxanthin with 12.0% lipid should show enhanced reproductive performance for both sexes of pond reared-broodstock *P. monodon*. Their compound diets composition are presented in Table 9.

The nutrient requirement of shrimp broodstock is not clearly understand. Several studies used compound diets in feeding trials. The nutritional composition of those diets are presented in Table 10. It can be concluded that the protein content should not be lower than 45% with at least 10% dietary lipid. Carotenoid especially, astaxanthin content, should be at least 250 mg/kg. The amount of HUFA should be considered. Table 11 presents the amount of AA, EPA and DHA and shows that essential fatty acid is necessary in shrimp broodstock diet.

#### 4.3 Broodstock feeding practices: Combination of fresh feed and compound shrimp diet

At present, for most *P. monodon* hatcheries, good reproductive performances are observed when shrimp broodstock are fed a mixture of natural feeds and pelleted feeds. The mix of fresh feed and the compound feed causes less reproductive disruption. The two portions of both diets varies. Recent studies recommended the feeding strategies for *P. monodon* broodstock as summarized in Table 12.

#### 5. Conclusion

Either wild or captive shrimp broodstock are able to develop sexual maturation under maturation diet feeding in a closed hatchery system. The fresh feed; polychaete, mollusk and squid are commonly used. Their nutritional value can

vary with species, season of collection and life stage. In addition, their use is labor intensive and significantly affects water quality. Development of compound diet has a benefit for the hatchery routine practice. However, the current knowledge on *P. monodon* broodstock nutrient requirement allows only using the mix of fresh feed and the compound diet without reproductive disruption. Future investigation on shrimp broodstock nutritional requirements and maturation diet will enable compound diets to be formulated in such a way as to allow the complete replacement of fresh feed. This will ensure a high larval production and prolonged sexual performance of shrimp broodstock in captivity.

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Table 9. Feed ingredients of *P. monodon* broodstock diet

Ingredients	Dry weight (g/100 g of diet)
Fish meal	56
Shrimp head meal	10
Wheat flour	16
Refined tuna fish oil	8
Chlorophyll pink	0.625
Cellulose	4.258
Mineral mixture	1
Vitamin mixture	1
Cholesterol	1
Lecithin	1
Binder	1
Vitamin A	0.04
Vitamin C	0.057
Vitamin E	0.02

Paibulkichakul *et al.* (2008)

Table 10. Proximate composition of compound diet for *P. monodon* broodstock

Component	Madmac <sup>1</sup> ♣ (Bio-Marine, Inc.)	Lucky Star <sup>2</sup> ♦ (Taiwan Hung Kuo Industrial Pty Ltd.)	Commercial Shrimp pellet <sup>3</sup>	HFHA <sup>4*</sup> ♣
Protein	52.3	61	48.6	48.8
Crude fat	14.2	13	15.1	12.0
Carbohydrate	15.7	-	-	13.86
Ash	10.2	-	-	12.40
Moisture	7.5	10.1	9.74	12.87
Astaxanthin (mg/kg)	-	-	-	264.6

<sup>1</sup>Yong Seok Kian *et al.* (2004); <sup>2</sup>Coman *et al.* (2007) <sup>3</sup>Meunpol *et al.* (2005); <sup>4</sup>Paibulkichakul *et al.* (2008)

♣ % as dry weight basis; ♦ % as fed basis

\*HFHA; high fish oil, high astaxanthin diet

Table 11. Highly unsaturated fatty acid (HUFA) profile of compound diet for *P. monodon* broodstock (% total fatty acid)

HUFA	Madmac <sup>1</sup> (Bio-Marine, Inc.)	CLO <sup>2*</sup>	HFHA <sup>3**</sup>
Arachidonic Acid (AA)	0.3	6.5	1.7
Eicosapentaenoic Acid (EPA)	4.0	11.8	7.1
Docosahexaenoic Acid (DHA)	13.2	6.2	19.2

<sup>1</sup>Yong Seok Kian *et al.* (2004); <sup>2</sup>Millamena (1989) <sup>3</sup>Paibulkichakul *et al.* (2008)

\*CLO; cod liver oil diet, \*\*HFHA; high fish oil, high astaxanthin diet

Table 12. The combination of fresh feeds and compound shrimp diet fed to shrimp broodstock.

Portions of fresh feed per compoundshrimp diet and ration size	Sources
Broodstock pellet 1-2% BW per day Mussel 15-30% BW per day	Quinitio <i>et al.</i> (1996)
Semi-moist diet 50% fresh feeds (squid, mussels, polychaete at ratio 1:1:1) 50% (32.5% squid : 32.5% bivalves : 5% polychaete: 30% pellets)	Meunpol <i>et al.</i> (2005) Coman <i>et al.</i> (2007)
HFHA pellet 2% BW per day Chopped squid ( <i>Loligo</i> sp.) 10% of BW per day	Paibulkichakul <i>et al.</i> (2008)

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