THE USE OF SEAWORM MEAL IN MATURATION DIET AS PARTIAL SUBSTITUTION OF FRESH DIET FOR POND REARED TIGER SHRIMP BROODSTOCK

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ABSTRACT

The purpose of this experiment was to evaluate the effects of using seaworm meal in artificial diet as partial substitution of freshfeed for maturation of tiger shrimp. This experiment started by growing-out tiger shrimp with initial weight around 60 g for four months until reaching maturation phase where shrimp weight were over 90 g for female. Tiger shrimp was selected and stocked into 10 ton concrete tank with stocking density of 50 shrimps with ratio of female : male of 1:1. Dietary treatments were different levels of seaworm meal at 0% (SW0), 10% (SW10) and 20% (SW20). SW0 was positive control without seaworm meal but breeder was fed with frozen seaworm. Test diets were fed as a combination of 60% test pellet and 40% fresh feed. Artificial insemination was carried out for all females before ablation to obtain fertile eggs. Results showed that after ablation, number of female matured was highest in group fed SW10 (13 breeders) and the lowest in female fed control group (7 breeders). Number of female spawned was also highest in female fed SW10 and the lowest was in positive control. Fecundity was very low in all treatments ranged from 12,000-79,700 eggs/spawn. Even though female bearing spermatophore through insemination, number of spawning hatched was very low, only three spawned in each of SW0 and SW10 and two spawned in SW20. Based on number of breeders matured and spawning rate, breeder fed with SW10 gave better performance than other two diets. Technique of artificial insemination needs to be improved to increase the number of fertile eggs.

KEYWORDS: polychaeta, maturation, pond-reared tiger shrimp

INTRODUCTION

Many studies have proved that nutrition has important role in reproduction of fish and crustacea (Kanazawa, 1985; Wouters *et al*., 2001; Racotta *et al*., 2003). One of important research that has been widely applied in hatchery around the world is the utilization of several kinds of fresh feed or combination between fresh or frozen natural diet and artificial diet which resulted in a better reproduction performances compared with feeding the breeder with only one type of fresh diet (Primavera *et al*., 1981; Chamberlain & Lawrence, 1981; Wouters *et al*., 2002).

The importance of using fresh feed for maturation process of shrimp is due to the fatty acid content of those diets in particular arachidonic acid (ARA, 20:4n6), eicosapentaenoic

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acid (EPA, 20:5n3), and docosahexanoic acid (DHA, 22:6n3) (Cavalli *et al*., 1997; Coman *et al*., 2007a). The main constraints of utilizing fresh feed in hatchery are that their nutrients contents are fluctuated and can increase the risk of bacterial and viral transmition (Harrison, 1990) specifically crustacea and seaworm. Hatcheries have already tried to apply artificial diet which majority consisted of combination of either moist or dried pellet in small portion (around 16%) and the rest is fresh feed (Wouters *et al*., 2000).

Domestication of tiger shrimp carried out by CSIRO Australia was divided into two rearing phase. The first eight months was grow-out phase and from 8th month to 11-12th month on was maturation phase. During maturation phase, shrimp are fed with 32.5% squid, 32.5% bivalves, 5% polychaeta combined with 30% maturation pellet (Coman *et al*., 2007a). Based on previous trial, it was found that combination of fresh feed and pellet diet at ratio of 40:60 resulted in a similar reproductive performances with 100% fresh feed and better performances compared with when breeder were fed with semi-moist diet (Laining *et al*., 2014). This indicated that during maturation stage, tiger shrimp can utilize artificial diet in large portion than fresh feed for gonad maturation process.

The role of polychaeta seaworm in reproductive system of penaeid shrimp in particular tiger shrimp is very important and it is claimed as the best feed for maturation process besides as amino acid and fatty acid sources, it is also suspected to contain active compound related to reproductive hormone (Lytle *et al*., 1990). Application of seaworm extract induced gonad maturation of tiger shrimp without ablation (Poltana, 2005). Several hormones have been identified in seaworm such as osmoregulatory hormone (Andreis, 2001), oxytocin/vasopressin hormone (Oumi *et al*., 1996 and Fujino *et al*., 1999), reproductive hormone (Meunpol *et al*., 2010), sex hormone (Hardege *et al*., 1994), sex pheromone (Zeeck *et al*., 1998) and immunehormone (Salzet, 2001).

Even though positive effect of seaworm on maturation process of crustacean has been intensively reported as a viral carrier (Meunpol *et al*., 2005; Binh *et al*., 2012), its use as a fresh feed remains to be a main problem. On the other hand, the use of seaworm meal as feed ingredient for artificial diet may reduce the viral transmission into the hatchery.

The objective of this study was to evaluate the effects of utilization of seaworm meal in artificial diet as partial substitution of fresh feed for maturation of tiger shrimp breeder in order to support domestication process both in pond and closed system tank.

MATERIALS AND METHODS

Rearing of Tiger Shrimp until Maturation Stage in Earthen Pond

This activity was conducted in experimental pond of Research and Development Institute for Coastal Aquaculture (RICA) in Laikang, Takalar, by rearing tiger shrimp with average initial body weight of 60 g (ranged from 40-80 g). Shrimp were stocked at around 12 months of age and the shrimp were cultured from larvae induced with faster growth marker. Shrimp were reared in 1,500 m^2 earthen pond with stocking density of 1.0 shrimp/m^2. During culture period, the shrimp were fed with commercial diet which contained around 45.4% crude protein (Table 1) enriched with vitamin C and carotenoid, fed at 2.5% of biomass. Shrimp were fed twice a day in the morning and afternoon. Fresh squid was fed once a week at approximately 5% of biomass in the morning. Growth was monitored every month by measuring weight and length of shrimp for four months or when female weight was > 90 g and male > 70 g which are categorized as maturation stage. Harvest was done at this phase and shrimp then transferred to controlled tank for adaptation. During adaptation period, shrimp were selected according to desired weight, healthy condition, normal genital organ, and then checked if female bearing spermatophore.

Feeding Trial for Maturation Stage in Controlled Tank

Following trial was carried out in hatchery of tiger shrimp of RICA in Barru. Dietary treatments were two levels of seaworm (SW) meal at 10% (SW10) and 20% (SW20). Control group was fed with diet without seaworm meal but with fresh/frozen seaworm. Diet was given according to feeding regime of 60% tested diet and 40% fresh feed. Fresh feeds being used for the feeding trial were squid and bivalves. Breeders were cultured in three tanks of 10 m^3 concrete tank with stocking density of 50 shrimp/tank and ratio of male and female was 1:1 (Coman *et al*., 2007b). Experimental diet was formulated based on previous studies on nutrient requirements for crustacean maturation

Table 1. Proximates analysis of commersial diet used during prematuration stage in earthen pond (%)

Nutrient	Percentage (%)
Dry matter	91.4
Crude protein	45.4
Lipid	8.1
Ash	12.4
Fibre	4.3

(Hoa, 2009; Marsden *et al*., 1997; Paibulkichakul *et al*., 2008; Wouters *et al*., 2001b) and also based on in-house formulation that has been previously evaluated (Laining *et al*., 2014). During feeding trial, water was supplied as flow-through system. Diets were given at rate of 2.5% of biomass, four times a day at 08.00; 12.00; 16.00, and 21.00.

For all tanks, females were eye-tagged for individual recognition and molt-tagged (water proof label glued to carapace and rubber attached to eye stalk) to identify when each female had molted. Molting of each female was monitored throughout trial. Females were unilaterally ablated two days after the first molt. Artificial insemination was carried out if natural mating did not happen during culture (Coman *et al*., 2007a).

Females were examined daily for ovarian maturation. Ripe females were transferred to 175 L circular spawning tanks, filled with around 150 L and allowed to spawn. After spawning or if the ovaries had regressed, females were weighed and returned to the maturation tanks. Fecundity per spawning was estimated from the total number of eggs collected in four 250 mL samples taken from the spawning tank water. Eggs were then allowed to hatch in the spawning tanks and naupli numbers per spawning were estimated with similar way of estimating the eggs number.

Biological parameters observed during maturation were survival and reproductive traits including natural gonad maturation stage of female or maturation after ablation, spawning

Table 2. Formulation of experimental diet (dried pellet, g/kg) with different levels of seaworm meal for maturation of tiger shrimp, *P. monodon*

Ingredients	SW0	SW10	SW20
Anchovy fishmeal	280	220	200
Mysid meal	160	180	130
Oyster meal	250	180	150
Seaworm meal	0	100	200
Wheat gluten	30	30	30
Rice bran	30	30	30
Wheat flour	100	100	100
Fish oil	45	45	45
Soy lecithin 70%	15	15	15
Cholesterol	2	2	2
Vitamin premix	30	30	30
Astaxanthine (Carophyll pink)	1.25	1.25	1.25
Vitamin C (Stay C)	0.57	0.57	0.57
Vitamin A and D	0.457	0.457	0.457
Vitamin E	0.2	0.2	0.2
Spirulina sp. (SP Green)	5	5	5
Mineral premix	30	30	30
Organic mineral	5	5	5
Attractant	2	2	2
CMC	13.527	23.527	23.527
Total	**1,000**	**1,000**	**1,000**

rate, fecundity, and hatching rate. Observation of sperm quality was also carried out by measuring the spermatophore weight, number of spermatozoa, and number of normal and abnormal sperm. Number of sperm was calculated according to Leung-Trujillo & Lawrence (1987). Spermatophore was homogenized using glass tissue grinder for three minutes, added with 3 mL Ca^{2+} free saline solution and then put into haemocytometer to calculate the sperm cell under microscope. To calculate number of normal and abnormal spermatozoa, 0.1 mL of sperm-saline suspension was added with 0.1 mL trypan blue dye, kept for 5-10 min. and then calculated under microscope. Minimum cell calculated was 100 cells according to Alfaro (1993).

Proximate analysis was done according to AOAC International (1999). Fatty acid analysis of freeze dried seaworm, test diets, and gonad were carried out using Gas Chromatography (Shimadzu 2010 plus, Tokyo, Japan). Amino acid analysis of seaworm was conducted using HPLC (Shimadzu 20A, Tokyo, Japan). Data on reproductive performances were presented descriptively.

RESULTS AND DISCUSSION

Fatty Acid Profile of Ingredients Used for Maturation Diet and Amino Acid Profile of Seaworm

Fatty acid profile of seaworm and several other ingredients used for maturation diet are presented in Table 3. The EPA and DHA of squid and oyster are relatively higher than mysid and seaworm. This data was also reported in the previous experiment except for seaworm (Laining *et al*., 2014). The high ARA content among ingredients were detected in seaworm and mysid around 2.82% (w/w in lipid) or 0.20% (w/w in ingredient). In addition, DHA, and EPA contents were relatively higher in all ingredients (w/w in ingredient) particularly squid and oyster compared to seaworm.

Profile of amino acid of freeze-dried seaworm is shown in Table 4. Generally, essential

Table 4. Proximate analysis and profile of amino acids of freeze-dried seaworm used in the experimental diet

Nutrient	Percentage (%)
Moisture	7.3
Crude protein	52.0
Lipid	6.2
Ash	15.7
Fibre	10.8
Aspartic acid	4.82
Glutamin acid	7.49
Serine	1.79
Histidine	0.91
Glycine	2.47
Threonine	1.56
Arginine	3.07
Alanine	2.82
Tyrosine	1.38
Methionine	1.17
Valine	2.05
Phenylalanine	2.39
Isoleucine	2.11
Leucine	3.34
Lysine	2.93

Table 3. Profile of fatty acids of several ingredients used in formulation of experimental diet (% of lipid)

Type of fatty acid	Mysid meal	Squid meal	Seaworm meal	Oyster meal
Fat content	2.61	7.54	7.22	9.36
Fatty acid:				
- Linoleic Acid, C18:3n6 (LOA)	1.59	6.87	1.28	1.08
- Linolenic Acid, C18:3n3 (LNA)	1.37	2.52	0.74	0.14
- Arachidonic Acid, C20:4n6	3.57	0.62	2.82	1.97
- Eicosapentaenoic Acid, C20:5n3	8.81	8.44	1.61	5.45
- Docosahexaenoic Acid, C22:6n3	14.85	12.67	0.14	9.73

amino acid content of seaworm was lower than anchovy meal but relatively similar to commercial brown fish meal (Hertrampf and Piedad-Pascual, 2000).

Fatty Acid Profile of Maturation Diets Used for Feeding Trial

Profile of fatty acids of maturation diets based on analysis are presented in Table 5. Concentration of linoleic and linolenic acid were relatively similar among test diets in a range of 0.31%-0.37% and 0.09%-0.10%, respectively. Similarly, EPA and DHA content were also the same among diets, however detected EPA level of the three diets (0.49%-0.56%) was lower than estimated. Concentration of ARA increased (0.16%-0.28%) as inclusion of seaworm meal in diet increased. Other nutrients required for maturation process such as vitamin and astaxanthine were also included in the diet at the same level.

Growth of Tiger Shrimp Reared in Earthen Pond

Survival rate of tiger shrimp after 120 days culture in pond was 55% and weight gain was 34.3%. Growth pattern during the culture is described in Figure 1. It showed that even though age of shrimp at stocking were already around 12 months its growth was still linear (Figure 1).

However, at the end of culture when shrimps were around 16 months of age, its stagnant growth was indicated by lower weight gain around 30%. In nature, shrimp grow rapidly until 9 months of age and then stagnant (Rothlisberg, 1998). Even though shrimp at harvest reaching 16 months of age, the survival rate was still high probably due to the positive effect of pellet with 45.4% crude protein combined with small part of fresh feed. Relatively higher survival rate obtained during pond culture also indicated that stocking density of 1 shrimp/m^2 was suitable for rearing tiger shrimp until maturation stage.

Further assessment showing that no female shrimp beared spermatophore in the thelicum when transferred to the maturation tank, implying that natural mating did not happen during culture in pond. This indicated that male shrimp can not spawn in pond environment. Coman *et al*. (2007a) reported that after feed-

Table 5. Nutrient composition of the test diets applied during maturation trial (%)

Nutrient	SW0	SW10	SW20
Dry matter	91.9	91.5	91.0
Crude protein	46.7	46.5	46.0
Lipid	9.4	9.7	9.9
Ash	12.1	11.7	11.5
Fibre	2.1	3.1	4.0
Energy (GE, MJ/kg)*	17.5	16.6	16.0
LOA (18: 2n-6)	0.31	0.37	0.33
LNA (18:3n-3)	0.09	0.10	0.09
ARA (20:4n-6)	0.15	0.17	0.28
EPA (20:5n-3)	0.56	0.56	0.49
DHA (22:6n-3)	1.19	1.14	0.9
Total n-3	1.90	1.81	1.54
Total n-6	0.62	0.57	0.52
Ratio n3/n6	3.06	3.18	2.96
Total phospholipid*	2.82	2.63	2.46
Cholesterol*	0.31	0.32	0.30
Vitamin C*	0.2	0.2	0.2
Vitamin E*	100 mg/kg	100 mg/kg	100 mg/kg
Astaxanthin*	106 mg/kg	105.4 mg/kg	104.2 mg/kg

* Values were estimated by nutrient content of each ingredient used database in a feed formulator

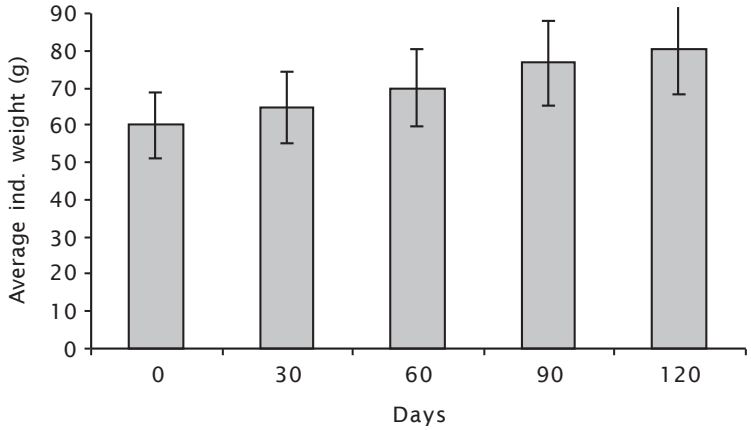

Figure 1. Average individual weight of tiger shrimp during 120 days reared in earthen pond

ing with two combination maturation diets, it was found only one out of 17 female of 2nd generation of tiger shrimp that matured naturally. It was fed with control diet consisting of 32.5% squid, 32.5% bivalves, 5% polychaetes (*Marphysa* sp.), and 30% pellet diet since shrimp aged 10 months until reproductive assessment held. These two findings indicated that low mating rate was still remain a problem in domestication of tiger shrimp both in pond and controlled tank (Coman *et al*., 2007a).

Reproductive Performances of Tiger Shrimp

Weight gain of female broodstock and survival rate of all broodstocks after four months feeding trial of maturation stage were presented in Table 6. Weight of female at the end of feeding trial increased little bit or even stagnant ranging from 3.8% to 8.4%. At this time, female broodstocks were around 20 months of age. Survival rate of broodstock (calculated for all female and male broodstock) was from 66%-70%, it showed that broodstock can survive longer in controlled tank if nutrition and other environment factors were in proper condition. This also indicated that at maturation stage, tiger shrimp broodstock can be reared longer without sand-bottom substrate.

Reproductive performances of pond reared breeders are presented in Table 7. During acclimatization and before ablation, no breeder matured naturally. Number of broodstock matured after ablation was the highest in group fed SW10 (13 breeders) and the lowest was in broodstock fed SW0 (7 breeders). Number of spawning was also the highest in group fed SW10 and lowest in SW0. All groups had lower fecundity ranged from around 12,000 to 79,000 eggs/spawn. Since none of female beared spermatophore during rearing period in pond, all females were artificially inseminated to produce fertile eggs. However, number of spawn that hatched was very low only three spawning for each of SW0 and SW10 and two spawning in SW20. Total nauplii (F2) of each

Table 6. Weight and survival rate of pond reared shrimp phase during four months maturation period in controlled tank

Parameters	SW0	SW10	SW20
Initial ind. weight of male (g)	73 (n = 25)	75 (n = 25)	73 (n = 25)
Initial ind. weight of female (g)	106 (n = 25)	107 (n = 25)	106 (n = 25)
Final ind. weight of female (g)	110	116	113
Weight gain of female (%)	3.8	8.4	6.6
Survival rate (male and female, %)	70	66	70

Table 7. Reproductive performance of pond reared shrimp phase maturation fed different levels of seaworm meal

Parameters	SW0	SW10	SW20
No. of naturally matured stock	0	0	0
No. of breeder maturing after ablation	7 (n = 24)	13 (n = 24)	11 (n = 24)
No. of breeder spawned per ablated stock	6	11	9
Range of fecundity (eggs/breeder)	12,300-79,700	12,500-67,165	14,000-65,500
No. of spawning that hatched (inseminated)	3	3	2
Total nauplii/tank (larvae/tank)	56,483 (n=3)	19,945 (n=3)	2,990 (n=2)
No. of 2nd rematuration (breeder)	4	3	6

tank were only around 56,000 larvae found in SW0 and 19,900 larvae in SW10.

Performances of male breeder at the end of feeding trial are shown in Table 8. Weight of spermatophore obtained by electrical shock was relatively similar among groups ranged from 0.04-0.05 g. Number of sperm per spermatophore was the highest at fish fed SW10, while shrimp fed SW20 had relatively similar sperm cell. Number of normal sperms ranged from around 70-78 (x 10^6 cells/spermatophore) which were relatively similar among groups. On the other hand, number of abnormal sperm ranged from 22 to 30 (x 10^6 cells/spermatophore).

Low natural mating rate found in this trial and other studies showed that mating rate remains the main constrain in domesticated tiger shrimp to produce broodstock. Hoa (2009) reported 17%-22% natural mating rate of tank reared domesticated tiger shrimp breeder which was relatively high that compared to what CSIRO domesticated team reported (Comen *et al*., 2005) and compared to the present trial which no natural mating found during the feeding period. Even though artificial insemination was carried out for all survived females, very low fertile eggs obtained in this trial implied that only few inseminated females could fertilize their eggs. Techniques of insemination applied on this trial needs to be modified to increase number of fertile eggs.

Low fecundity found in this trial was in the range of eggs fecundity of domestiscated tiger shrimp reported by other works. Comen *et al*. (2005) found that egg fecundity of several families of pond reared tiger shrimp breeder ranged from 56,260-167,710 eggs/broodstock, 11-17 months of age. These shrimps were fed with combination diets consisting of 20% squid, 5% bivalves, and 70% high protein pellet until eight months of age and from 8 to 11 months of age fed with 30% squid, 20% mussels, and 5% polychaetes combined with 45% pellet.

Domesticated tiger shrimp reported by Hoa (2009) showed a variety of egg fecundity ranged from 60,000-617,000 eggs/spawning with average of 309,630 eggs. Maturation diets that were applied on their study were also

Table 8. Reproductive performance of pond reared male maturation phase fed different levels of seaworm meal

Parameters	SW0	SW10	SW20
Weight of male (g)	87.2	74.4	72.0
Age of male breeder (months)	20	20	20
Weight of spermatophore (g)	0.05 (n = 4)	0.04 (n = 4)	0.05 (n = 3)
No. of sperm/spermatophore (cell x 10^6)	63.8	91.5	66.8
No. of normal sperm (cell x 10^6)	75.5	78.1	69.7
No. of abnormal sperm (%)	24.5	21.9	30.3

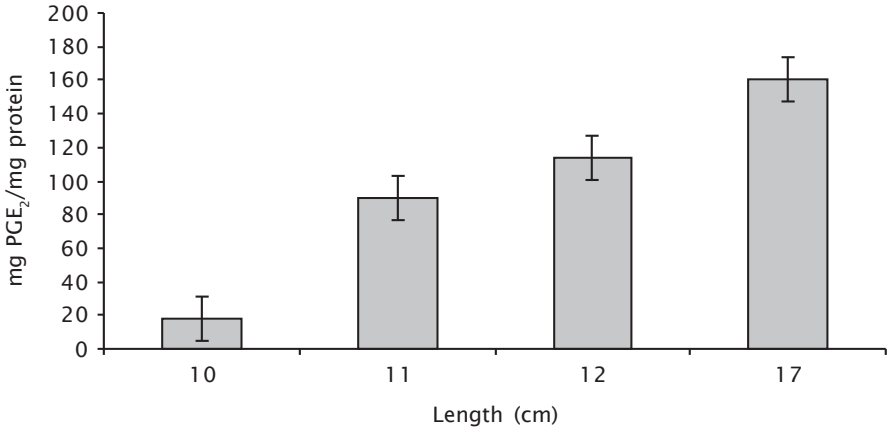

Figure 2. Levels of prostaglandin E_2 (PGE_2) measured in natural sand polychaetes, *Perinereis* sp. at size differences (Meunpol *et al*., 2010)

combination between 40% fresh feed (37% squid, 27% oyster, 17% seaworm, and 19% pig liver) and 60% pellet.

Among natural fresh feed that were extensively used for gonadal maturation and spawning of penaeid shrimp, the best is squid and polychaete seaworm (Yano, 2000; Meunpol *et al*., 2005). The role of these two diets on the reproductive process has not been fully understood, but many studies reported that squid and seaworm are rich in fatty acids content such as n-3 HUFA and PUFA specifically EPA, DHA, dan ARA (Wouters *et al*., 2001a dan Racotta *et al*., 2003). This is in line with fatty acid analysis of main ingredients used in this trial which confirmed the high EPA and DHA in squid and high ARA content in seaworm meal. Besides their fatty acids content, marine polychaetes also contained some sexual steroids hormones such as 17β-estradiol, progesterone, prostaglandin in *Nereis virens* (Garcia-Alonso & Rebscher, 2005), *Nereis diversolor* (Mouneyrac *et al*., 2006). Recent studies reported that *Perinereis* sp. contained several reproductive hormones including progesterone which were effective in enhancing the final maturation and 17α-OHP4 had more effects on vitellogenic oocytes (Meunpol *et al*., 2007). Moreover, prostaglandin E_2 (PGE_2) was also detected in polychaete as shown in Figure 2 which enhanced oocytes development especially during late development and ovulation (Meunpol *et al*., 2010).

Steroid-like hormones in marine polychaetes also possess some amines and/or neuropeptides including osmoregulatory hormone, sex pheromones (Hardege *et al*., 2004), oxytocins involved in regulating reproductive functions such as mating recognition and gamete release (Andries, 2001). Binh *et al*. (2012) tried to extract polychaete into different fractions and found that polychaete extract in form of NLF (neutral lipid fraction) gave the most effective fraction on ovarian maturation of *Marsupenaues japonicus* broodstock and followed by TSF (trichloroacetic acid soluble extract). They assumed that NLF contains some active substances for ovarian maturation and TSF may contain these polychaete amines/neuropeptides that might be directly or indirectly involved in sexual maturation of *M. japonicus*.

CONCLUSION

Based on number of matured female and spawned female as well as male reproductive performances, it is concluded that 10% of seaworm meal was enough to be included in maturation diet. Artificial insemination is highly required to allow more assessment of reproductive performances in order to evaluate the effect of maturation diet on tiger shrimp breeder.

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