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Impact of Dietary Protein Levels on the Reproductive Performance of *Penaeus monodon*

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Abstract: The effects of dietary protein levels on reproductive performance of eyestalk ablated and non-ablated *Penaeus monodon* were studied using a series of diets containing 30, 35, 40, 45 and 50% protein levels. Significant differences among diet treatment were observed in the total number of spawning and fecundity. The ablated females spawned much more times than non-ablated females. Ablation was found to shorten the latency period between ablation and first spawning. The first spawners were from ablated females, depicting lower survival rate when compared to non-ablated female and male.

Key words: Dietary protein levels % Ablated and non-ablated females % *Penaeus monodon* % Reproductive performance

INTRODUCTION

Successful technologies for induced maturation and spawning of viable eggs of prawn are available in India for two principal species namely *Penaeus indicus* and *Penaeus monodon*. *P. monodon* is considered to be the most suitable species because of its higher growth and survival rate [1]. As the potential of penaeids have been realized, they are cultured intensively. *P. monodon* is difficult to breed, requiring unilateral eyestalk ablation of maturation in captivity [2]. Removal of the eyestalk by ablation eliminated inhibitory hormones (OIH and MIH) and leads to maturation or moulting depending on which stage of the molt cycle, ablation is performed. Although, ablation has been widely used to induce maturation, a decline in hatch rate [3] and an increase in partially developed ovaries and partial spawning were also observed. This type of reproductive decline may be attributed to an over stimulation of spawning [4] or decrease in sperm quality and quantity [5]. To improve egg and larva quality, recent studies on maturation of penaeid shrimp have focused on the nutritional requirements of the brood stock during ovarian development. Diet may play a significant role in the survival of eyestalk-ablated decapods [6].

Proteins are considered to be the most expensive fraction and indispensable nutrient for growth and maintenance of life of all animals [7]. The knowledge of the protein requirement of a species is essential in the formulation of well-balanced and low cost artificial diet [8].

Most of the previous authors fed ablated penaeids with fresh mussel flesh, frozen shrimp [4], frozen artemia [9] and frozen squid [10]. In general, fresh or fresh-frozen diets currently used for maturation are high in marine animal protein, approximately 60% with amino acid profiles similar to that of shrimp protein. Only a handful of authors took interest on formulation artificial maturation diet in the form of pellet. Primavera and Caballero [10] fed the brood stock with a maturation pellet containing 52.8% crude protein at 2% body weight every afternoon in combination of live annelids or squid at 5% body weight in the morning. In 1992, they used 52% crude protein diet in combination of fresh frozen squid. Primavera *et al.* [3] assessed the effect of brown mussel meat and chopped squid and their combined effects on reproductive performance of ablated *P. monodon.* The protein level of their pelleted diet was 43.5%. There was wide variation on the reported values of dietary protein requirements of ablated and non-ablated brood stocks of penaeid prawns.

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The lack of clearly defined nutritional requirement for eyestalk-ablated prawn has been considered as an obstacle to develop a well-balance diet. Studies on the allocation of energy for somatic growth, egg production under conditions of restricted food supply in intact and ablated prawns are still meagre. Though, eyestalk ablation technique is widely practiced to enhance growth and reproduction of prawns, no attempt has so far been made to study the interaction of ration and ablation [11].

The purpose of the present study was to evaluate the effects of dietary protein levels on the reproductive performance of eyestalk ablated and non-ablated *P. monodon.*

MATERIALS AND METHODS

Experimental Animals and Tank: *P. monodon* procured from Bay of Bengal near Tuticorin, were placed in stocking tank under ambient temperature and salinity. Six prawns were reared in each tank for five different diets. Parallel experiments were conducted for both eyestalk ablated and non-ablated prawns. The stock has mean body weight of 106 ± 5.38 gm for females and 60 ± 3.49 gm for males and stocked at a ratio of 1:1 into the tank with two meters in diameter and the sea water was re-circulated through a filter. Two third of the water in the tank was exchanged every week and the water flow through the re-circulating system was exchanged every week and it was adjusted to 1-2 l/min. Each tank was provided with continuous aeration by an air stone connected to the air pump.

Diet: Five experimental diets (Table 1) were formulated to contain protein contents ranging from 30-50% with an increment of 5% protein. Sunflower oil and cellulose contents were used to maintain constant energy levels. The diets were prepared by thoroughly mixing the dry ingredients with oil and the adding water until stiff dough was produced. Then the dough was pelletized through a 4 mm die and oven dried to approximately 8% moisture content at 50°C for 12 hr. The diets were analyzed for dry matter (105°C, overnight) crude protein (Kjeldahl’s method), lipid [12] and ash content (550°C, overnight).

Table 1: Proportion of different ingredients (9/100g) and proximate composition (%) of diets.

Ingredients	P1	P2	P3	P4	P5
Fish meal	10.0	10.0	10.0	10.0	10.0
Shrimp meal	10.0	10.0	10.0	10.0	10.0
Soyabean meal	5.0	5.0	5.0	5.0	5.0
Squid meal	10.0	10.0	10.0	10.0	10.0
Casein	15.8	21.7	27.6	32.9	39.0
Cod liver oil	6.3	6.3	6.3	6.3	6.3
Starch	4.5	4.5	4.5	4.5	4.5
White dextrin	29.1	22.6	26.2	10.4	4.0
Supplevite -m	3.0	3.0	3.0	3.0	3.0
Nacl	0.5	0.5	0.5	0.5	0.5
Sodium benzoate	4.8	5.4	5.9	6.4	6.7
Cellulose	4.8	5.4	5.9	6.4	6.7
Proximate composition					
Calculated protein	30.0	35.0	40.0	45.0	50.0
Estimated protein	29.6	34.3	39.4	45.3	49.2
Crude lipid	9.9	7.8	7.3	6.2	5.3
Carbohydrate	25.3	23.4	20.0	17.7	15.4
ME (Kcal/100g)	308.7	301.0	303.3	307.8	306.1
P:E	95.9	113.9	129.9	148.1	160.7

ME= Metabolizable energy content, based on physiological fuel values of 4.0, 4.5 and 9 kcal/g of carbohydrate, protein and fat respectively.

P: E = Protein to energy ratio in mg protein/ kcal of ME.

Supplevite -M

Vitamin A 50,00,000 IU Vitamin B2 2.0 gm Calcium 750g

Vitamin D3 10,00,000IU Vitamin E 750 units Vitamin K 1.0 gm

Vitamin B12 6.0 mg Nicotinamide 10 gm Copper 2.0 gm

Calcium panthothenate 2.5 gm Manganese 27.5 Cobalt 0.45 gm

Choline chloride 150 gm Iodine 1.0 gm Iron 7.5 gm

Zinc 15gm

Eyestalk Ablation: The eyestalks of females were unilaterally ablated during the intermoult period. An electrocautery apparatus was used to perform the ablation operation, preventing bleeding and infection [13]. They were randomly stocked for experiments with five different diets in equal proportion.

Two experimental groups were defined as eyestalk ablated and non-ablated. Each group was further divided into five groups for five protein diets. They were fed with respective protein diet at four percent of their biomass per day. The feed was given twice daily, half ration in the morning and the other half in the afternoon for a period of 63 days. Unfed and faecal matters were removed twice daily before feeding. Water temperature and salinity were measured during the experimental period and they were in the range of 27°C to 37°C and 28 to 33 ppt respectively. Seawater used in the spawning tanks was settled for at least 24 hrs prior to use.

Evaluation of ovarian development was made to know the degree of maturation. The ovary of *P. monodon* could not be seen easily through the thick-pigmented cuticle. Hence, they had to be caught every 2-3 days a week and examined against a bright source of light to determine the degree of ovarian development. When the females were about to have fully developed gonad they were transferred individually for spawning in 100l spawning tank containing filtered seawater. Spawning took place either on the same right or ion the subsequent night. After spawning, the females were removed and the number of eggs spawned and the number of hatched nauplii was estimated as follows; six samples each was 100 ml of eggs were taken for the determination of fecundity and percent hatch rate. Three samples were used for fecundity and other for percent hatch rate. The eggs were counted, taking into account of total water volume of the spawning tank. The average was used to estimate the number of eggs produced per female. A similar procedure was used to determine the percent hatch rate.

RESULTS

Table (2) shows the stocking data of ablated and non-ablated *P. monodon*. The first females to spawn were the ablated ones, followed by non-ablated. Fecundity, number of nauplii per spawn and percent hatch rate differed within diet treatments and between ablated non-ablated prawns. The non-ablated females took longer time to mature and sometime did not mature. The ablated prawns took 20 to 28.5 days for getting maturity depending upon the supply of varied dietary protein levels. The shortening in maturation time led to an increase in the number of spawns. But, the non-ablated prawns took 42 to 50 days to attain maturity.

Tables (3 and 4) show the reproductive performance of non-ablated and ablated prawns, respectively, fed on varying dietary protein levels. The ablated brood stock spawned more times and produced more eggs than the non-ablated ones. Thus, ablation increased the performance by five times the total number of spawns. The highest first and subsequent spawning was from ablated groups fed on diet containing 40% protein level. Fecundity increased regardless of ablation. It was good at 40% protein level in ablated prawns (1,15,600 eggs/spawning) and it was at 35% protein level in non-ablated prawns (90,600 eggs/spawning).

Table 2: Stocking data for ablated and non-ablated P. monodon fed under different protein levels.

	Protein levels (%)				
	30	35	40	45	50
a, Non-ablated					
Carapace length (mm)	5.96±1.6	58.8±1.0	60.1±1.2	58.0±1.6	60.6±1.1
Body weight (gm)	108.6±1.0	112.8±7.2	107.1±9.6	113.6±6.8	108.8±3.8
b, Ablated					
Carapace length (mm)	58.2±1.2	58.6±1.2	59.4±1.2	60.3±1.6	59.2±2.1
Body Weight (gm)	107.0±7.6	106.8.1±7.6	110.1±6.6	112.6±6.6	110.1±7.8
Male					
Carapace length (mm)	46.2±1.4	45.8±1.8	46.1±1.5	46.6±1.8	45.9±1.6
Body weight (gm)	59.6±3.6	56.8±2.2	58.9±2.2	60.1±4.6	57.6±3.6
Duration	63 Days				
Male : Female ratio	1:01				

Table 3: Spawning, fecundity and hatching rate of non –ablated P. monodon fed different protein levels.

	Protein levels (%)				
	30	35	40	45	50
No.of. Spawning					
No. of. Spawning	----	1	1	-----	1
First					
Second	------	-----	------	------	------
Third	------	-----	-----	-----	-----
Total	------	1	1	-----	1
Total no of eggs produced	------	90,600	84,200	-----	82,800
Average. No. of eggs/spawning	----	90,600	84,200	----	82,800
Total no of nauplii produced	----	28,300	18,800	---	20,400
Percentage of hatch	----	31.23	22.33	----	24.64
Remarks	*I	*2	*2	*1	*2

*1 Underdeveloped ovary; *2 Weak nauplii; metamorphosis –very low; discarded

Table 4: Spawning, fecundity and hatching rate of ablated P. monodon fed with different protein levels.

	Protein levels (%)				
	30	35	40	45	50
No. of. Spawning					
First	2	2	4	2	3
Second	------	1	1	1	1
Third	-----	----	-----	-----	-----
Total	2	3	5	4	4
Total No. of Eggs produced	1,77,300	2,78,00	6,93,800	4,27,400	4,06,300
Average. No. of eggs/spawning	88,600	92,800	1,15,600	1,06,800	1,01,600
Total no of nauplii Produced	---	66,400	2,09,200	1,12,400	1,07,300
Average. No. of nauplii/spawning	----	22,100	34,900	29,100	28,800
Percentage of hatch	---	23.82	30.19	26.31	28.35
Remarks	*1	*2	*2	*2	*2

*1 Underdeveloped ovary; *2 Weak nauplii; metamorphosis –very low; discarded

Table 5: Latency period (in days) between ablation and first spawning and successive spawning of ablated P. monodon.

	Protein levels (%)				
	30	35	40	45	50
No. of. Spawning					
No. of days between ablation to first spawning	28.5	22	20	21	20
First to second spawning	-----	8	6	8	7
Second to third spawning	------	----	4	4	-----

Percent hatch rate on the basis of average number on nauplii hatched per spawning was 22.33 to 31.23% for non-ablated and 23.82 to 30.19 % for ablated prawns. However, the total number of nauplii hatched was maximum in ablated prawns at 40% protein level (2,09,200 nauplii) but it was only 28,300 nauplii in non-ablated prawns fed at 35% dietary protein. The percent hatch rate for ablated female was high (30.19%) at 40% protein level.

Non-ablated females required a long time for maturation and spawning when compared to ablated females (20 to 28.5 days). The shortening in maturation time led to an increase in the number of spawns.

The successive spawning time was as short as 4 to 6 days for 40% protein diet and 4 to 8 days for 45% protein level (Table 5). There was no subsequent spawning in non-ablated prawns. High mortality was observed across the diet treatment and throughout the experimental period. Mortality was higher in females than in males. Among females, mortality was higher in ablated prawns when compared to non-ablated females. The survival of ablated females, ranged from 22.22 to 33.33% and in unablated females it was 33.3 to 55.56%.

DISCUSSION

The ablated prawns took only 20 to 28.5 days for maturation whereas the non-ablated females took longer time up to 50 days for maturity. This is probably due to the lower blood titre value of ovary inhibiting hormones (OIH) [14]. Removal of the eyestalk by ablation eliminates the inhibitory action of OIH and leads to maturation. Kelemec and Smith [15] found that the unablated females took much longer time to develop their ovaries in *P. plebejus*. Similar observation was made by Emmerson [4] in *P. indicus*. Emmerson [4] has stated that the ablated *P. monodon* had their ovarian development after 22 days of ablation, but unablated females took about 65 days. Unilateral ablation is found to reduce the spawning period. Primavera [2] was able to get first spawning from ablated *P. monodon* in 22 days after ablation. Whereas, Halder [16] had the first spawning in ablated prawn only after 38 days of ablation.

All the previous findings indicate that the ablation causes a six-fold increase in the spawning performance of penaeid prawns. In the present study, the highest spawning performance was recorded from ablated groups of prawn fed on diet containing 40% dietary protein, but there was no difference in number of spawning in the case of non-ablated prawns. Similarly, it was also reported that the process of ablation can increase the reproductive rate of *P. indicus* by more than six times of the non-ablated prawns [3, 20]. The ablated *P. semisulcatus* is found to give more number of spawns and eggs than those of non-ablated females [9]. Hillier [17] obtained maturation and spawning from ablated *P. monodon* over a period of 75 days but not in unablated ones. Fecundity is high in ablated females (88,600 to 1,15,600 eggs/spawning) when compared to non-ablated females (82,800 to 90,600 eggs/spawning). In the present study, the total number of nauplii produced were high in ablated prawns at 40% protein level (2,09,200 nauplii/spawning) but it was only 28,300 nauplii in non-ablated females fed on 35% protein level. The percent hatch rate was 30.19% for ablated females fed with 35% dietary protein. The hatch rate is often affected by progressive spawning. There appears to be no over stimulation of spawning in non-ablated females and hence they have a better hatch rate [5].

Mortality was higher in ablated females when compared to unablated females and males. The higher survival rates of non-ablated females and males could be due to lack of stress from ablation, handling and frequent spawning. However, the survival rate non-ablated and ablated females in the present study are very poor compared to previous reports [10, 18-20]. The present study clearly indicates that protein levels have significant effect on the reproductive performance of ablated and non-ablated *P. monodon*.

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