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SUBSTITUTION OF FISHMEAL PROTEIN BY SOYBEAN MEAL IN DIET FOR GIANT FRESH WATER PRAWN *MACROBRACHIUM ROSENBERGII* (de Man)

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Abstract: A 95 days experiment was conducted to observe the growth performance of giant fresh water prawn (*Macrobrachium rosenbergii*) replacing fishmeal protein by dietary soybean meal protein in ten earthen mini ponds (50 m^2), at Khulna University campus . Five experimental diets containing 25% protein as Control, Diet 1, Diet 2, Diet 3 and Diet 4 were used. In Control, 36% fishmeal was used as a sole source of protein and Diet 1, 2, 3 and 4 were prepared by substituting fishmeal protein with 30, 40, 50, and 80% fishmeal protein with soybean meal protein respectively. Juvenile shrimps were collected from local vendors having 1.7 g average weight and stocked at a density of 3 m^{-2}. Water and soil quality was monitored regularly and found within the acceptable range. The prawns were fed twice daily at satiations by serving the diets in feeding trays. The highest average weight gain was found in Diet 4 (23.89 ± 5.29 g) and the lowest in Diet 2 (17.68 ± 1.09), although no significant difference ($p>0.05$) was observed among the treatment and the lowest FCR (1.09) the highest SGR (2.60) and PER (3.43) was achieved in Diet 4 that showed significant difference ($p<0.05$) with that of Control. Survival rate in Control, D1, D2, D3 and D4 was found 71%, 83%, 95%, 95% and 86% respectively. Among all treatments the better growth performance was found in Diet 4.

Key words: Giant freshwater prawn, *Macrobrachium rosenbergii*, feed, protein substitution, soybean meal

Introduction

Economically productive aquaculture systems depend upon an adequate supply of low-cost feeds with high nutritional quality. Feed represents up to 55 - 60 % of total operating cost in shrimp culture (Akiyama *et al*., 1992). Fishmeal has traditionally been used as a major ingredient in fish feed preparation because of its high quality protein and palatability (Lovell, 1984). The global supply of fish-oil and fishmeal is relatively limited. The increasing demand, high cost and uncertain availability of this finite item leads the nutritionists to search for alternative sources to compensate high valued fishmeal (Barlow, 1989). Therefore, an increasing interest in partial or complete replacement of dietary fishmeal by other protein sources, animal or plant, in aquaculture feeds has been grown. However, among feedstuffs of plant origin soybean meal is considered the most

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nutritive, being widely utilized in fish diets. The target of this research was to optimize the substituted level of dietary soybean meal to fishmeal.

Materials and Methods

***Pond study*:** The study was conducted in ten earthen mini ponds, with a water depth of 1 m and 50 m^2 surface area. Excavation, predator elimination and liming were done to maintain the water level, water quality and to protect the extruder in to the ponds. Juvenile *M. rosenbergii* were stocked at 3 m^{-2} at the end of July 2003. The experiment was carried out from August to November, 2003. Before stocking all the prestocking measurements like pond repairing, liming and removal of predator were done.

Selection and analyses of feed ingredients: Locally available feed ingredients (Table 1), considering both protein supplement and price were selected. The ingredients were analyzed at FMRT lab to determine the level of crude protein, crude lipid and ash according to AOAC (Anon, 1984).

Table 1. Proximate composition of different feed ingredients (dry-weight basis).

Feed ingredients	Protein %	Lipid %	Ash %	Cost (Tk. kg^{-1})
Fish meal	42.75	8.09	35.03	31.70
Soybean cake	52.25	2.61	6.20	17.00
Wheat flour	15.74	2.15	3.12	10.40
Wheat bran by product	19.00	5.21	4.20	8.40
Rice polish	14.81	-	14.10	6.20
Oyster shell	-	-	-	2.50
Shark oil	-	-	-	50.00
*Binder	-	-	-	180.00

**Binder (PEGABIND®) a proprietary blend of synthetic resins derived from modified urea and formaldehyde that assist in manufacturing process by formation of molecular chains and cross-linking condensation reaction, Bentoli, Inc. USA*

Feed formulation and feeding: In the experiment, five different diets (the Control and the 4 other diets) were formulated by using 'Pearson square' method (De Silva and Anderson, 1995) using a locally made pelleter powered by electricity (Table 2). Four experimental feeds were prepared by replacing fish meal protein by soybean meal as follows - D1: 30% replacement, D2: 40% replacement, D3: 50% replacement; D4: 80% replacement. Feed was given 2 times a day, early in the morning (before sunrise) and just after sunset. Initially feed was given 5 to 7 % of the total body weight by using feeding tray placed 18-20 cm above the pond bottom.

Table 2. Inclusion of different ingredients in feed formulation (in kg, dry weight basis).

Feed	Fish meal	Soybean cake	Wheat flour	Wheat bran by product	Rice polish	Oyster shell	Shark oil	Binder	Dry wt. basis % Lipid	Dry wt. basis % Protein	Cost (Tk. kg^{-1})
Control	36	-	17	21	20	3	3	0.006	25.14	8.98	20.75
D1	25.25	8.83	17	21	21.67	3	3.25	0.006	25.28	9.61	18.65
D2	21.6	11.78	17.2	21	21.92	3	3.5	0.006	25.51	8.02	18.02
D3	18	14.72	17.7	21.16	21.92	3	3.5	0.006	25.7	10.08	17.31
D4	7.2	23.56	18.33	21.66	22	3	4.25	0.006	26.8	8.04	16.33

Variable measured: Growth, survival, SGR (Specific Growth Rate), and FCR (Feed Conversion Ratio) were evaluated. Proximate analyses of the experimental feed were done following standard procedures (Anon, 1992). Water quality parameters like temperature, pH, dissolved oxygen, alkalinity, soil pH and total nitrogen was measured. DO (Winkler method, determined with Azide modification of iodometric method (Anon, 1992) and temperature were measured two times a day at 5:00 and 17:00 h for the duration of culture period. Water pH (pH meter Woon Socket, HANNA) and alkalinity (measured with titrimetric method) were measured once a week at 9:00 h and soil pH and total nitrogen were measured fortnightly.

Statistical analysis: The growth responses of the prawn were calculated for each pond (mean body weight, biomass, growth rate, survival, food intake, food conversion, protein intake and protein efficiency ratio (PER) and were subjected to analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) (Steel and Torrie, 1988).

Results

The mean values for initial weight, weight gain, SGR, PER, FCR, survival rate of *Macrobrachium rosenbergii* fed different treatment feeds for 95 days experiment are summarized in Table 3. The mean final weight of the prawn varied from 19.38 to 25.59 g. The highest average weight of prawn was observed in the group Diet 4 (25.59 g) followed by the Control (20.11 g) and the lowest was observed in Diet 2 followed by Diet 1 and Diet 3 (19.38 g, 19.57 g and 19.92 g respectively). No significant difference in weight observed using different diets. In case of FCR no significant difference was observed among the treatments except the Control and Diet 1 that varied significantly ($p<0.05$) with the others. The lowest PER was observed in Control that varied significantly ($p<0.05$) with Diet 4 which showed the highest (3.43). No significant difference was observed in SGR among the prawns of Control, Diet 1, Diet 2 and Diet 3. However a significant difference ($p<0.05$) in SGR was observed between Control and Diet 4. The lowest survival rate was observed in Control (71.33%) that showed no significant difference ($p<0.052$) with Diet 2 and Diet 3.

Table 3. Growth performance of prawn with different diets.

	Feed (replacement of fish meal protein by soybean meal)				
Parameters	Control	D-1	D-2	D-3	D-4
Initial weight (g)	1.7 ± 0^{a}	1.7 ± 0^{a}	1.7 ± 0^{a}	1.7 ± 0^{a}	1.7 ± 0^{a}
Weight Gain(g)	$18.41 \pm.45^{a}$	17.87 ± 5.4^{a}	17.68 ± 1.09^{a}	18.23 ± 1.35^{a}	23.89 ± 5.29^{a}
SGR(%./day)	2.17 ± 0.09^{a}	2.29 ± 0.19^{ab}	2.43 ± 0.12^{ab}	2.46 ± 0.12^{ab}	2.60 ± 0.10^{b}
PER	2.23 ± 0.1^{a}	2.42 ± 0.19^{ab}	3.06 ± 0.65^{ab}	2.93 ± 0.38^{ab}	3.43 ± 0.34^{b}
FCR	1.79 ± 0.01^{c}	1.64 ± 0.13^{bc}	1.30 ± 0.28^{a}	1.34 ± 0.18^{ab}	1.09 ± 0.11^{a}
Survival rate (%)	71 ± 8^{a}	83 ± 9^{ab}	95 ± 6^{b}	95 ± 5^{b}	86 ± 6^{ab}

Data are the mean values and SD. Values within the same row having different superscripts show significant difference ($p<0.05$).

All prawns of each pond were weighed initially to categorize them into different weight groups (Grade). Six categorize of prawn (Grade ≤U-70, U-60, U-50, U-30, U-20 and U-10; U-70=60 to 70, U-60=50 to 60, U-50=40 to 50 whole prawns comprise of 1 kg weight.) were done and Table 4 shows the percent occurrence of prawn in different grades. Highest percentage was ≤U-70-grade for all treatment .The ≤U-30 grade varied significantly ($p<0.05$) with other grade.

Table 4. Percentage of occurrence of prawn by different weight group categories (grade).

Feed	≤U-70	U-60	U-50	U-30	U-20	U-10
Cont	$44.05^{a}\pm6.58$	$12.58^{a} \pm0.65$	$31.59^{a} \pm5.32$	$7.66^{ab} \pm3.45$	$4.13^{a} \pm1.55$	.00
Diet 1	$50.59^{a} \pm13.52$	$9.49^{a} \pm2.54$	$28.59^{a} \pm4.29$	$7.97^{ab} \pm3.45$	$3.37^{a} \pm3.70$	.00
Diet 2	$50.74^{a} \pm1.53$	$12.42^{a} \pm6.18$	$27.12^{a} \pm5.31$	$5.55^{a} \pm1.65$	$3.84^{a} \pm.27$	.34±. 47
Diet 3	$44.65^{a} \pm2.93$	$10.32^{a} \pm5.03$	$34.20^{a} \pm7.06$	$7.06^{a} \pm1.38$	$3.80^{a} \pm2.28$	.00
Diet 4	$32.45^{a} \pm9.05$	$5.82^{a} \pm0.09$	$33.47^{a} \pm4.55$	$21.00^{b} \pm8.33$	$7.26^{a} \pm5.18$	.00

Values are the mean and ±SD. same superscripts in the same column indicate no significant difference ($p>0.05$).

The mean values for water quality parameters like water pH, alkalinity, temperature, dissolved oxygen, soil nitrogen and soil pH are summarized in Table 5. No significant difference (p>0.05) of mean temperature between morning and the evening, dissolved oxygen, alkalinity, soil nitrogen and soil pH of the ponds was observed in the in the experiment.

Table 5. Physioco-chemical parameter of water and soil of the experimental ponds.

	Different Feed Treated Ponds				
Parameters	Control	D-1	D-2	D-3	D-4
Water pH	7.7 ± 0.14ª	7.65 ± 0.21ª	7.65 ± 0.07ª	7.65 ± 0.07ª	7.7 ± 0.00ª
Water Alkalinity	345 ± 54ª	372 ± 85ª	402 ± 4ª	343 ± 14ª	307 ± 8ª
Temp Morning [1]	27.8	27.8	27.8	27.8	27.8
Range	(31 – 20)	(31 – 20)	(31 – 20)	(31 – 20)	(31 – 20)
Temp Evening[1]	31.5	31.4	31.4	31.5	31.5
Range	(35.4 - 21)	(35.4 - 21)	(35.4 - 21)	(35.4 - 21)	(35.4 - 21)
DO Morning[1]	2.76	2.47	2.71	3.02	2.95
Range	(6 - 1)	(5.8 - 1.1)	(6 – 1.3)	(6.3 – 1.2)	(5.6 – 1.4)
DO Evening[1]	6.82	7.29	7.92	7.28	7.10
Range	(9.90 – 4.3)	(11.1 – 4)	(12.7 – 4.4)	(9.8 – 4.1)	(9.3 – 4.15)
Soil N	0.16 ±0.04ª	0.19 ± 0.07ª	0.17 ± 0.05ª	0.19 ± 0.03ª	0.16 ± 0.01ª
Soil pH	7.2 ± 0.11ª	6.91 ± 0.27ª	7.12 ± 0.08ª	6.99 ± 0.13ª	7.14 ± 0.05ª

Values are the mean and SD. Different superscripts in the same row show significant difference (p< 0.05).

Discussion

Physico-chemical parameter of soil and water

Temperature: Freshwater prawns are considered tropical animals requiring relatively high temperatures (29-31 °C) for maximum growth (New, 1995). Many authors reported that the *Macrobrachium rosenbergii* do the best in water of 22–32 °C (Daniel, 1981). Adult prawns are tolerant to a wide range of temperature from 18 to 34 °C but the best condition for reproduction is thought to be 28°C (Chavez Justo *et al*., 1991). However, no detrimental affect of high temperature was observed on the prawns in the present experiments and temperature was within acceptable limit.

Dissolved oxygen (DO): A DO concentration above 4 mg l^{-1} during grow out period is essential to avoid metabolic stress on prawn (Hall and Van Hamm, 1998). The mean values of DO among the ponds receiving different diets in the morning (2.47-3.02 mg l^{-1}) did not show any significant difference. Jia-Mo *et al.* (1988) reported the DO at 4.5 mg l^{-1} in *M. rosenbergii* culture ponds. Hossain *et al*. (2000) reported the DO content at 3.0-6.1 mgl^{-1} in earthen ponds in monoculture of *M. rosenbergii*. Hossain *et al.* (1989) reported fluctuation in DO in prawn culture pond between 4 and 4.70 mg l^{-1}.

pH: Prawns need somewhat alkaline water, pH 7.2 to 8.4 (Daniel, 1981). Hossain *et al*. (1989) reported the pH of prawn nursing pond between 7 and 8.4. Michael (1969) suggested the best pH for fish at 7.5-8.5. It is best to avoid a pH below 6.5 or above 9.5, if possible (Boyd, 1990). New (1995) reported that *M. rosenbergii* can be reared at optimal pH range 7.0- 8.5. The acceptable pH level for *M. rosenbergii* is 6.2-7.4 based on growth and feeding (Chen and Chen, 2003). Hossain *et al.* (2000) reported that pH ranged from 6.8 to 8.4 was suitable for *M. rosenbergii.* The water pH in the present experiment was slightly alkaline (pH 7.6-7.8) with a fluctuation level of pH 6.9 to 8.2 within 95 days of study period. The water pH value of the experiments was within acceptable limit.

Soil pH: Banerjea (1967) suggested that for several fish species, optimum soil conditions are as follows: pH: 6.5–7.5; available phosphorous 60 ppm as P_2O_5; available nitrogen: 250–750 ppm; and, organic carbon: 1.5–2.5%.The mean soil pH in the experimental ponds was observed to be slightly alkaline (pH 6.91-7.2) with a fluctuation levels of 6.5- 7.4 and available nitrogen varied from 0.16 to 0.19 % with a fluctuation levels of 0.13- 0.31 %. The soil pH of this experiment was observed within acceptable limit but the available nitrogen value of the experiment slightly higher than the optima.

Alkalinity: Chisty and Rahman (1999) reported a total alkalinity between 100 and 175 mg l^{-1} as good indicator of water quality for *Macrobrachium rosenbergii* culture in gher farming system. Boyd (1990) recommended alkalinities of above 20 mg l^{-1} as $CaCO_3$ for fresh water prawn. The mean water alkalinity in the experimental ponds was 307-402 mg l^{-1} as $CaCO_3$ which varied from 252 to 486 mg l^{-1} during the 95 days experiment. The results of the above studies more or less show that the pH and alkalinity of the present study was slightly higher than optima for *M rosenbergii*. However, no adverse affect on the growth of *M. rosenbergii* was observed throughout the experiment.

Growth: Pongmaneerat and Watanabe (1993) succeeded in utilizing 30% SBM (Soybean meal) as a replacement of fish meal in an extruded high energy diet for small rainbow trout of 5 g initial weight. Lim and Dominy (1990) reported that 40% of the marine protein mix could be replaced by solvent-extracted soybean meal; however, higher levels of replacements resulted in reduced growth in shrimp feeds. Gallagher (1994) found that the replacement of up to 75 % of fishmeal protein with SBM as possible in the diet of hybrid striped bass with methionine supplements. With marine fish species, to date, the maximum successful inclusion of dietary SBM in practical marine shrimp diets is not more than 50%, as higher levels resulted in lower growth (Lim and Dominy, 1990).

A similar study was conducted on juvenile prawn *Macrobrachium rosenbergii* by Du and Niu (2003). Prawns were fed diets where 0%, 20%, 50%, 75% and 100% of fishmeal was replaced by soybean meal. Feed intake was not significantly affected by inclusion of SBM. Standard metabolic rate was significantly affected by dietary soybean level, and was the highest in the 75% SBM group. They concluded that SBM, without supplementation of amino acids or other additives, was not suitable as a major protein source in freshwater prawn diets. The results of the present study partially differ with the findings of Du and Niu (2003). But the following statements could be used in support of the present study. Natural productivity is a significant food source in prawn production (Lilyestrom *et al*., 1987). Prawns are able to adjust to the absences of feed pellets by increasing consumption of available vegetation (Weidenbach, 1980). Prawn may be able to adjust to reduction the nutritional value of prepared diets (i.e. protein source and vitamin and mineral content) by increasing predation on natural fauna (i.e. macro vertebrates) in the pond (Tidwell *et al*., 1995).

The quality of soybean meals are known to vary, however, owing to growing conditions, cultivars, storage conditions and production practices (Snyder and Kwon, 1987). Therefore, the present results cannot be generalized for all soybean meals, but are valid only for soybean meal qualities similar to the one used in this experiment. The inclusion of soybean meal in fish diets has produced contradictory results mainly because of different heat treatments used to inactivate possible anti-nutritional factors from this feedstuff, e.g., antitrypsin activity. (Robaina *et al*., 1998)

The use of SBM is technically limited by its amino acid composition compared to fish meal (FM) (deficiency in the essential amino acids methionine, lysine and tryptophan), presence of antinutritional factors, and poor palatability (Lim and Dominy, 1990). On the other hand, heat treatment of soybeans improves the growth performance and feed utilization in trout (Sandholm *et al*., 1976), common carp (Nour *et al*., 1989), and coho salmon (Arndt *et al*., 1999). Viola *et al.* (1983) reported that heating SBM at 105 ^{o}C for 30–90 min destroyed most of the protease inhibitors. However, heating may cause loss of essential amino acids (Plakas *et al*., 1985).

The fishmeal that was used in the present studies was purchased from local market in Khulna. The storage condition was not hygienic and some insect infestation took place at storage and from proximate analysis (Table 1) it is assumed that it may reduces the protein availability. It was also noticed by personal interview with the entrepreneurs that fishmeal produced from the fish (Bombay duck, *Harpodon nehereus*, a fatty fish) was dried in the sun. Fatty acid oxidation could have take place during sun drying which may affect its quality and subsequently the growth of prawn in the control treatment.

Survival rate: Chisty and Rahman (1999) achieved 80-93% survival of prawn fed low cost feed in gher farming system at stocking density of 3 m^{-2}. Wills and Berrigan (1977) recorded a survival rate of 50% as acceptable in farming practice. Daniels *et al.* (1995) reported the highest survival rate at 73.7-81.9% for *M. rosenbergii* fed with a specially formulated diet in earthen ponds. Tidwell *et al.* (1993) reported 78% survival rate in the monoculture of *M. rosenbergii* using formulated diet containing 32% protein. The survival rate (71–95%) in the present study was much higher than the above studies.

FCR: Hossain *et al*. (2000) reported that the FCR values of diets ranged from 3.06 to 4.85 where prawns were fed with 32 % protein and the production ranged between 304.5 and 563.3 kg ha^{-1} in 105 days. Comparatively better FCR, PER and SGR was achieved with Diet 4 in the present experiment. Diet 4 showed significant differences ($p<0.05$) in FCR, PER and SGR with the control diet.

Stocking density: Stocking densities vary greatly depending on the farming system and individual management practices. In extensive farming systems the stocking density is usually maintained at 1-3 shrimp m^{-2}, while in semi-intensive, intensive and super intensive system it can be 10, 50 and 160 shrimp m^{-2} respectively (Liao and Chien, 1994). Thangdurai (1991) reported that a stocking density of 3 m^{-2} was optimum in India feeding on a combination of groundnut cake, rice bran and fish flesh. Hossain *et al.* (2000) stocked 2.5 prawns m^{-2} (*M. rosenbergii*) to observe growth using low cost feed ingredients. The culture of *M. rosenbergii* in Brazil is generally practiced through semi-intensive system, with a stocking density at 5-10 prawns m^{-2}. Yield was varied from 1000 to 2500 kg ha^{-1} year $^{-1}$ (Valenti, 1993). The stocking density in the present study was 3 m^{-2} with a mean yield of 601 kg ha^{-1} for diet 4 in 95 days.

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Conclusion

Diet 4 (80% SM+20% FM) was found as the best for *Macrobrachium rosenbergii* in earthen ponds considering SGR, PER and FCR. While Control (100% FM) gave an inferior result. Rather than 100% fishmeal, a combination of fishmeal and other supplemental source of protein give better result for *M. rosenbergii* in earthen pond monoculture system.

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