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## Effect of Alkali-Soluble Fraction of Paddy Husk and an Experimental Low-Cost Feed on Growth of *Macrobrachium rosenbergii*

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### ABSTRACT

The effect of a low-cost diet was evaluated in field trials on the length and weight performances of *Macrobrachium rosenbergii* for more than a 12-month period. The experimental diet was evaluated against a commercially available prawn feed and was seen to give statistically significant better performances regarding length and weight of *Macrobrachium rosenbergii* post-larvae at all periods of study. At the end of the study period, adult prawns exhibited more than 46% higher weight when fed with the experimental diet compared to commercial diet-fed prawns. It was further observed that prawn post-larvae performs better if in the initial six weeks of growth the diet is supplemented with an alkali-soluble fraction obtained from paddy husk. Cumulatively, the results suggest that cultivators can benefit in lowering both feed costs and obtaining bigger size prawns if they use the experimental diet.

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## INTRODUCTION

*Macrobrachium* constitutes about 70% of the total freshwater prawn production in Bangladesh. The predominant species cultivated is *Macrobrachium rosenbergii* (English: giant freshwater prawn, Bengali: golda chingri). Agroecological conditions favor the cultivation of this prawn species and at present more than 100,000 households now practice integrated rice cum prawn and fish farming. This is in addition to the cultivation of giant freshwater prawn by itself in water bodies like ponds or “ghers”, which is done mostly in the south-western districts of Bangladesh alongside districts like Noakhali and Gopalganj. The current area under cultivation has been estimated to be around 30,000 hectares, and it is increasing by about 20% each year.

One of the biggest problems at present facing cultivators of *Macrobrachium rosenbergii* are several diseases, which although have not reached endemic proportions yet, are still strong enough to cause financial losses. The second is the high cost of feed. Although the causative agents for different diseases have not been well-studied in Bangladesh, a perusal of the data available from other countries gives an idea of the diseases, since the same symptoms have been reported from cultivators of this species in Bangladesh. *Aeromonas veronii* and *Aeromonas caviae* have been isolated from the hepatopancreas of apparently healthy giant freshwater prawns in Taiwan (Sung *et al*, 2000). Novobiocin and vancomycin-resistant *Aeromonas* spp. have also been isolated from gills, swimmerets, eggs, stomachs and ventral muscles of *Macrobrachium malcolmsonii* available in the local fish market of Dhaka, Bangladesh (Rahim and Aziz, 1994). *Enterococcus* infection, which causes depression in immune system of the prawn, is also prevalent, and which is aggravated by low contents of dissolved oxygen in water (Cheng *et al*, 2002). Prawns have been known to suffer from epizootic yeast and bacterial co-infection. The bacterium has been identified as *Enterococcus faecium*, while the yeast has been identified as *Metschnikowia bicuspidata* (Chen *et al*, 2003). A bacterium species secreting two chitinolytic enzymes, *Chitinomonas taiwanensis*, has also been found in surface water of freshwater ponds for giant prawn culture (Chang *et al*, 2004). A viral disease (white tail disease) of the giant freshwater prawn, which leads to whitish appearance of muscles was first identified in some provinces of China, but has since spread to other parts of the world including India, Thailand and possibly Bangladesh. The causative agents have been identified as *Macrobrachium rosenbergii* nodavirus (MrNV) and an extra small virus (XSV) (Qian *et al*, 2003;

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Yoganandhan *et al*, 2006; Hsieh *et al*, 2006; Wang *et al*, 2007). A disturbing feature of the white tail disease is that it can be vertically transmitted from brooders to progeny (Sudhakaran *et al*, 2007).

Since treatment of diseases of the giant freshwater prawn is difficult, one approach that can be taken is the use of immunomodulators to boost up the natural defense system of the prawns. For instance, vitamin E has been incorporated in the diet to modulate antioxidant defense system (Dandapat *et al*, 2000). ImmuPlus, a polyherbal commercial formulation has also been tested to modulate the immune system of the giant freshwater prawn (Kumari *et al*, 2004). Another immunomodulator, which has recently gained attention from researchers is beta-1,3-glucan (henceforth referred to as glucan), usually extracted from yeast cell walls but is also present in the bran of cereals like wheat, barley, oat, and rye. Glucan, obtained from the culture filtrate of *Sclerotinia sclerotiorum* IFO 9395 has been shown to potentiate the immune response of mice following oral administration (Suzuki *et al*, 1989). Dietary beta-glucan also enhanced non-specific immunity and increased disease resistance in the catfish, *Clarias batrachus* when challenged with *Aeromonas hydrophila* (Kumari and Sahoo, 2006). Beta-1,3-1,6-glucan obtained from yeast cell wall extract induced non-specific disease resistance in the tiger shrimp, *Penaeus monodon*. Shrimps, which have been immersed in glucan or administered glucan in diet showed increased disease resistance to *Vibrio vulnificus* and viral agents extracted from the white spot syndrome virus (Song *et al*, 1997). Oral administration of beta-1,3-glucan derived from *Schizophyllum commune* in diet enhanced survival, haemocyte phagocytosis and superoxide anion production in brooder *Penaeus monodon* and further improved immunity and survival of shrimp, when challenged with white spot syndrome virus (Chang *et al*, 2000; Chang *et al*, 2003). It has also been reported that maternally transmitted disease resistance induced by glucan (derived from baker's yeast *Saccharomyces cerevisiae*) also protected the larvae of *Penaeus monodon* against white spot syndrome virus infection (Huang and Song, 1999).

Glucan has not been reported in paddy husks, which is widely available in Bangladesh. Following milling of paddy to obtain rice, the resultant portion (containing mainly husk with some bran) is usually discarded or added to poultry houses to absorb poultry excreta. Interestingly, while a lot of scientific attention has focused on brans from different cereals, husks have been more or less scientifically neglected. However, paddy husk is known to contain lignins. Immunomodulatory activities of cacao lignin-carbohydrate complex have been reported (Sakagami *et al*, 2011). We decided to investigate the effects of an alkali-soluble fraction obtained from paddy husk and a low-cost experimental feed on growth performances in the giant freshwater prawn, *Macrobrachium rosenbergii*.

## MATERIALS AND METHODS

### *Extraction of alkali-soluble fraction from paddy husk (ASFPH):*

ASFPH was extracted from paddy husk procured locally. Briefly, husk was boiled in 5 volumes of 1N NaOH at 90°C for 1h. The NaOH extract was cooled to room temperature, filtered and the filtrate acidified with HCl. The filtrate was stirred and left at room temperature, following which the precipitate that formed was collected, washed thrice with deionized distilled water and once with absolute ethanol. The precipitate was next dried in an oven at 60°C. The resultant product amounted to about 5-10% w/w of the starting material.

### *Preparation and maintenance of "ghers" (ponds):*

The experimental gher consisted of an area of 150 X 80 feet, equivalent to approximately 27.5 decimals. The experimental gher was surrounded by three control ghers, equivalent to 66, 66, and 132 decimals (100 decimals = 1 acre). All ghers were dried, fertilized, and limed prior to start of experiment utilizing standard procedures of the area. Liming was conducted every four months throughout the duration of the experiment. The ghers were localized at Kachua Thana, Pinguria Union of Bagerhat District. Regular changes of water were conducted every week, where fresh water was introduced from an adjoining canal. All ghers had a water level of approximately 2.5 feet. The water condition was mainly salty throughout the duration of the experiment except during the monsoon season (June to August). The whole experiment was conducted between September 2011 and November 2012. Dissolved oxygen and pH of the water was also monitored regularly. During the course of the cultivation period, dissolved oxygen ranged between 8.7-9.6 ppm and pH ranged from 7.1-7.8 in both control and experimental ghers.

### *Collection of *Macrobrachium rosenbergii* post-larvae (PL) and addition to ghers:*

10-day old post-larva were collected from a local dealer, who in turn collected them from natural sources. The PL had an average weight of 0.057g at the time of collection. PL was added to ghers on September 1, 2011 at a density of 1,000 PL per 27.5 decimals.

### *Diet:*

During the first 15 days of cultivation, PL in control ghers received a diet composed of flour, banana and eggs, while PL in the experimental gher received the same diet supplemented with 0.1% ASFPH. For the next 30 days, PL in control ghers received a commercial shrimp Nursery diet obtained from a local commercial fish

feed-producing Company (QFL) in Bangladesh (9.92% moisture, 36.86% crude protein, 17.82% ash, 0.52% acid-insoluble ash and 12.88% fat); during the same time period, PL in the experimental gher received the same diet supplemented with 0.1% beef and 0.1% ASFPH. After a total of 45 days, control gher prawns received a commercial shrimp Starter diet obtained from QFL (9.98% moisture, 36.78% crude protein, 19.02% ash, 0.57% acid-insoluble ash and 6.89% fat). Following another 30 days and then throughout the course of the experiment, control gher prawns received a commercial shrimp Grower diet obtained from QFL (9.52% moisture, 39.62% crude protein, 20.54% ash, 0.25% acid-insoluble ash and 8.23% fat). Beginning from the 46<sup>th</sup> day of cultivation and then throughout the duration of the experiment, prawns in the experimental gher were fed a diet composed of 80g maize, 10g molasses, 10g starch and 10g bone meal (6.33% moisture, 8.90% crude protein, 2.13% ash, 0% acid-insoluble ash and 2.04% fat). Prawns were sampled at regular intervals, their length and weight measured, and diet was given at 3% of the average weight of the prawns. The composition of various diets was obtained from the sample analysis report at the Bangladesh Animal Resource Center, Farmgate, Dhaka. The chart below shows the time period of feeding various diets to control and experimental gher prawns.

Time period	Diet given to prawns in control gher	Diet given to prawns in experimental gher
02-09-11 – 17-09-11	Composite diet (0.5 kg flour, 4 bananas, 2 eggs - smashed and mixed thoroughly prior to administration)	Composite diet (0.5 kg flour, 4 bananas, 2 eggs along with ASFPH at 0.1% of total weight smashed and mixed thoroughly prior to administration)
18-09-11 – 18-10-11	QFL Nursery Diet	QFL Nursery Diet supplemented with 0.1% beef and 0.1% ASFPH
19-10-11 – 19-11-11	QFL Starter Diet	Experimental diet (80g maize, 10g molasses, 10g starch and 10g bone meal)
20-11-11 – 29-11-12	QFL Grower Diet	Experimental diet (80g maize, 10g molasses, 10g starch and 10g bone meal)

Prawns from both control and experimental ghers were harvested on 02-12-2012.

## RESULTS AND DISCUSSION

Table 1 shows the length and weight of prawns from both control and experimental ghers collected during different periods of cultivation. By the end of the first 45 days of the cultivation period, prawns in the experimental gher fed a commercial diet supplemented with ASFPH and beef were significantly greater in length ( $P > 0.001$ ), as well as weighed more than prawns sampled from control ghers. This trend persisted till about 75 days following release of PL in ghers. The second sampling conducted after 73 days of cultivation (by this time prawns in the experimental gher were fed with the experimental diet) demonstrated that prawns in the experimental gher were more than double in weight and averaged nearly 50% greater length than prawns in the control ghers. The differences were statistically significant ( $P > 0.001$ ). The third and fourth samplings conducted during the months of December 2011 and January 2012, respectively, showed an overall decrease in the weight of prawns in both experimental and control ghers. It is to be noted that these samplings were conducted during the winter months, when due to the decrease of water temperature, aquatic species have reduced metabolism and as such, decrease in weight. However, even in the third and fourth samplings, prawns in the experimental gher exhibited greater lengths and weights than prawns from the control ghers, even though the differences were not statistically significant. The weight of the prawns from all ghers showed increases with corresponding increases in their lengths from March 2012, when the fifth sampling was done. In this sampling, a statistically significant increase in the length and weight of prawns from experimental gher was observed ( $P > 0.1$ ), when compared to prawns obtained from the control gher. The final sampling, done on December 2012 again demonstrated increases in both prawn weights and lengths as well as statistically significant increases ( $P > 0.02$ ) in both length and weight of prawns from the experimental gher versus prawns from control ghers.

Overall, it may be concluded that both the initial diet (incorporating ASFPH) and the latter experimental diet showed improved performances in increasing the length and weight of prawns compared to a commercially available diet. The results are suggestive of a two-fold benefit; one a possible increase in the innate immunity due to feeding of ASFPH, and second- the low cost of the experimental diet.

Several groups have attempted to develop artificial diet for prawns, which can sustain good growth (Kanazawa *et al*, 1970; Cowey and Forster, 1971; Deshimaru and Shegino, 1972, Sick *et al*, 1972; Andrews *et al*, 1972; Balazs *et al*, 1973; Forster, 1976). However, these studies did not deal with diet of *Macrobrachium rosenbergii*. Various studies have given different results for optimal protein concentration in giant freshwater prawn diet. A diet containing more than 35% protein has been proposed by Balazs *et al* (1973) for giant freshwater prawns. The growth performance for *Macrobrachium rosenbergii* PL has been found to be equally good with 40 or 49% protein in diet, while 23 or 32% protein showed depressed growth rates; however, after a cultivation period of ten weeks, when prawns averaged 1.15g, the 40% protein level diet gave better cumulative weight gain (Millikin *et al*, 1980). Using four commercial diets containing 18, 24, 24 and 49% protein levels

derived from pig starter, poultry starter, gamebird feed and trout chow, respectively, Fujimora and Okamoto (1970) did not find any appreciable differences in the average daily increase in length of juvenile *Macrobrachium rosenbergii*. In another experiment, soybean-tuna diet was observed to give the best growth for giant freshwater prawns followed by soy-tuna-shrimp diet, both being at 35% protein level (Balazs and Ross, 1976). No significant differences in growth rate for giant freshwater prawns were observed by Boonyaratpalin and New (1980) in an experiment in concrete ponds using 15, 25 and 35% protein diets as well as broiler starter for feed. It has been suggested that protein level of 25% or possibly less produce acceptable results for cultivation of *Macrobrachium rosenbergii* (New *et al*, 1980). Consistent with the above report, Bartlett and Enkerlin (1983), in a study conducted in asbestos asphalt bottom ponds using hard water (1,000 ppm) showed that a 14% protein diet within a granulated chicken feed gave comparable growth and survival of giant freshwater prawns as compared to other studies. On the other hand, in a recent study conducted in Bangladesh for 3 months in which *Macrobrachium rosenbergii* was monocultured, it was observed that a 30% protein diet consisting of 20% fish meal, 10% meat and bone meal, 15% mustard oilcake, 15% sesame meal, 35% rice bran, 4% molasses and 1% vitamin-mineral premixes gave optimal growth of the giant freshwater prawn (Hossain and Paul, 2007). It is to be noted that the study was conducted for only 3 months in closed ponds provided with aeration during the night using air pumps.

Our results indicate that a diet consisting of approximately 9% protein is capable of sustaining growth and length better than a commercially available diet consisting of more than 35% protein. In this aspect, our results are more in agreement with the results of Boonyaratpalin and New (1980) and Bartlett and Enkerlin (1983), whose studies indicated that a high protein diet is not essential for culture of giant freshwater prawns. The other thing that is to be noted is that the culture system used in the present study included changing of water in the ghers with water from an adjoining canal every week. The influx of water from the open canal could have been a source of nutrients including protein sources, leading to the acceptability of low protein in the diet of the prawns. The presence of only 9% protein also led to lowering of the manufacturing cost of the experimental feed (since feed proteins are costly items) without compromising the weight and length of the prawns, which was itself the focus of the present study.

**Table 1:** Length and weight of prawns sampled at different periods of cultivation.

Date of sampling	Sample number	Length <sup>a</sup> in cm (Mean ± SEM)		Weight in g (Mean ± SEM)	
		Control	Experimental	Control	Experimental
13-10-2011	13	1.65 ± 0.07	2.26 ± 0.12*	0.75 (total weight)	1.38 (total weight)
12-11-2011	10	6.42 ± 0.53	9.08 ± 0.29*	2.10 ± 0.41	5.15 ± 0.49*
15-12-2011	11	7.06 ± 0.52	7.83 ± 0.71**	3.29 ± 0.54	4.86 ± 1.24**
16-01-2011	5	7.70 ± 0.81	8.12 ± 0.74**	3.42 ± 1.06	4.28 ± 1.09**
24-03-2011	5	9.58 ± 0.75	11.48 ± 1.25**	7.38 ± 2.39	14.16 ± 4.25**
02-12-2012	30	17.66 ± 0.40	19.53 ± 0.58***	48.99 ± 5.36	71.44 ± 7.64***

<sup>a</sup>Every sample was measured from rostrum to telson during length measurement.

\*P<0.001

\*\*P>0.10

\*\*\*P<0.02

The manufacturing cost of the experimental feed used in the present study was more than 40% less than the commercial feed given to the prawns in the control ghers. Most of the cultivators of prawn are poor farmers. With the rising costs of prawn PL, any reduction in cost of feed can translate itself into a substantial saving and as such, substantial profit for the cultivator. The low-cost diet as formulated by Hossain and Paul (2007) also costs much more to manufacture than the experimental diet used in this study. Farmers thus can benefit in two ways- reduction of feed cost (which alone can account for more than 30% of the total cultivation costs) and at the same time not be dependent on imported feed material (like meat and bone meal), which is not only costly but also not available to the farmers at all times.

The initial addition of ASFPH to PL diet has the additional advantage of possibly conferring immunity in the proposed experimental cultivation of *Macrobrachium rosenbergii* in Bangladesh. However, the latter needs to be experimentally verified. In this connection, it may be mentioned that immunization of fish with plants or plant-derived products is not uncommon. The immunostimulatory effect of hot water extract of *Gracilaria tenuistipitata* on the white shrimp *Litopenaeus vannamei* and its consequent resistance against *Vibrio alginolyticus* has been reported (Hou and Chen, 2005). The plant, *Achyranthes aspera* has been shown to stimulate immunity in both *Catla catla* as well as the common carp, *Cyprinus carpio* (Chakrabarti and Vasudeva, 2006; Chakrabarti and Rao, 2012).

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