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Effect of dietary source and level of chitin on growth and survival of post-larvae *Macrobrachium rosenbergii*

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Summary

A 45-day feeding trial was conducted to study the effect of dietary chitin, either from a natural source (shrimphead meal) or in purified form, on the growth and survival of post-larvae (PL) *Macrobrachium rosenbergii*. Three hundred uniform size (average weight 20 ± 0.05 mg) PL were equally divided in five treatments, each with three replicates. Five isonitrogenous (35% crude protein) semipurified diets were prepared by varying the source and level: T_1 (5% purified chitin), T_2 (10% purified chitin), T_3 (5% natural chitin, equivalent to 22% shrimphead meal), T_4 (10% natural chitin, equivalent to 44% shrimphead meal) and T_0 as control (no chitin). The addition of chitin either from shrimphead meal (natural source) or in purified form enhanced the growth rate of PL significantly ($P < 0.05$) over the control group. No significant variation ($P > 0.05$) was observed in T_1, T_3 and T_4 groups with respect to weight gain percentage, specific growth rate (SGR) and feed conversion ratio (FCR). However, the growth rate of T_2 was significantly ($P < 0.05$) lower and similar to the control group. This suggests that PL *M. rosenbergii* were unable to utilize purified chitin at the 10% level in their diet. However, the 10% chitin equivalent of shrimphead meal (44%) did not show any growth depression. There were no chitinoclastic bacteria found in the gut of *M. rosenbergii* PL in any treatment group. The conclusion is that chitin can stimulate the growth of the *M. rosenbergii* PL. Shrimphead meal is a good source of chitin, which can be safely used in up to 44% of the diet.

Introduction

Macrobrachium rosenbergii is widely distributed in the Indo-Pacific region and is a popular species for aquaculture in India, Thailand, Taiwan, Malaysia, Indonesia, Bangladesh and other countries. A hardy species, it exhibits a high growth rate and is low in protein requirements compared with penaeid species. Metabolically required by most crustaceans, chitin is usually obtained through consuming either insects or other crustaceans. It is commonly observed that shrimp consume exuviae following ecdysis (Fox, 1993). Chitin is also present in crustacean faeces, and coprophagy is known to be important in shrimp (Frankenberg and Smith, 1967). Chitin of arthopod origin occurs naturally as a protein–polysaccharide complex and contains various lipids, pigments and minerals (Cocklin, 1982; Horst, 1989). Thus the natural chitin found in shrimphead meal may be of great nutritional value to shrimps.

Chitinase activity can be enhanced in the presence of native protein (Lindsay, 1984; Danulat, 1986a,b). Kitabayashi et al. (1971) demonstrated that the addition of 0.53% glucosamine in the diet improved the growth of *Masupenaeus japonicus*, but that growth was retarded when chitin was added to the diet. Akiyama et al. (1992) recommended a minimum dietary level of 0.5% chitin in shrimp feed. Fox (1993) observed no growth rate improvement in *Penaeus monodon* fed with diets containing 0%, 4%, 8%, 12% or 16% chitin, and concluded that chitin was not directly utilized by *P. monodon*. However, Clark et al. (1993) reported that shrimp could digest and assimilate chitin as a nutritional contribution.

Several studies have reported measurable levels of chitin-degrading enzymes in the alimentary tract of various crustaceans (Chandrmohan and Thomas, 1984; Lynn, 1990; Spindler-Bath et al., 1990). Hood and Meyers (1973) found that the chitinase enzyme is produced by chitinolytic bacteria in the gut of *Litopenaeus setiferus* and is directly related to the chitin content of feed. Fox (1993) reported with a chitin increase in the diet of *P. monodon* that the number of chitinoclast bacteria decreases. Chitinase is a complex of endo- and exoglucosidase produced either by gut chitinoclastic bacteria or by endogenous gut secretion (Goodrich and Morita, 1977a,b; Kono et al., 1990; Spindler-Bath et al., 1990). Chitin is degraded by exo- and endochitinase (Spindler, 1983; Kramer et al., 1985; Chen, 1987). Penaeid shrimp possess a gut microflora capable of producing chitinase (Hood and Meyers, 1973; Dempsey and Kitting, 1987). New (1976) stated that shrimp may be able to assimilate chitin through the activities of their gut bacteria.

Shiau and Yu (1998) observed that the addition of 5% purified chitin in the diet enhances *P. monodon* growth. But natural chitin could enhance the growth of *P. monodon* with the presence of lipid, protein, pigments and minerals (Fox, 1993). Comparative studies of both chitin sources, however, are lacking in *M. rosenbergii*. Hence, the purpose of this study was to elucidate the effect of dietary chitin, either in purified or natural form, on the growth and survival of *M. rosenbergii* post-larvae (PL).

Materials and methods

Experiments

Post-larvae *Macrobrachium rosenbergii* were obtained from the freshwater prawn hatchery of the Central Institute of Fisheries Education, Mumbai, and acclimatized to experimental conditions for a 2-week period before the start of the experiment.

Experimental design

The experiment was set up with five different experimental groups using three replicates each in 15 uniform-size plastic

tanks (75 L capacity) arranged in a completely randomized design. Twenty PL (average wet weight 20 ± 0.05 mg) were stocked in each tank. Continuous aeration was provided to all tanks to maintain optimum dissolved oxygen (DO). Faecal matter and leftover feed were removed manually and about 75% of the water was exchanged on alternate days with chlorine-free borewell water. Physico-chemical water parameters (DO, pH, temperature, CO_2, NH_4^+–N and NO_2^{--}–N) were measured as per the method of APHA-AWWA-WEF (1998). The DO level ranged from 7.68 to 8.40 ppm and temperature was within the range of 29–30°C. Free carbon dioxide concentration was negligible in all tanks throughout the experiment. Water pH varied from 7.6 to 8.4. The ammonia nitrogen level was below 0.50 ppm and the nitrite nitrogen level below 0.01 ppm in all tanks. PL were fed to satiation with their respective diets. About 1/3 of the ration was given at 10.00 hours and the remaining 2/3 at 19.00 hours. PLs were weighed at 15-day intervals and the daily ration was adjusted accordingly. The study duration was 45 days.

Experimental diets

Five semipurified isonitrogenous diets (Table 1) of 500 g each were prepared with a supplementation of 5% or 10% chitin either in purified or natural form, i.e. from shrimphead meal; the control group was without supplementation: T_0 (control), T_1 (5% purified chitin), T_2 (10% purified chitin), T_3 (5% natural chitin equivalent to 22% shrimphead meal) T_4 (10% natural chitin equivalent to 44% shrimphead meal). Shrimphead meal used in the experiment contained 23% chitin and 40% protein. Hence, to supplement 5% or 10% natural chitin, 22% or 44% shrimphead meal was supplemented respectively. Purified chitin was procured from Sd-fine Chemicals Ltd (Mumbai, India). All ingredients except the gelatin, vitamin mineral mixture, vitamins B and C were thoroughly mixed. Gelatin crystals were mixed in lukewarm water to form a gel; other ingredients were then mixed with the gelatin to form a dough. Oil was mixed in thoroughly and the dough was kept for 1 h for proper conditioning followed by steaming for 5 min at 15-psi pressure. The vitamin mineral mixture, vitamins C and B complex were mixed after cooling. Pellets of 2 mm were prepared using a hand pelletizer. The pellets were air dried followed by oven drying at 60°C overnight and stored in airtight polythene bags at 4°C until used.

Growth parameters

Post-larvae were weighed at 15-day intervals. They were starved overnight before taking body weight measurement. The growth indices were calculated as follows: FCR is the quantity of dry feed given per wet weight of experimental animal; SGR $= (\ln W_f - \ln W_i)100/t_2 - t_1$, where W_f is the final mean weight, W_i is the initial mean weight and $t_2 - t_1$ = duration of experiment (45 days); protein efficiency ratio (PER) is the weight gain per protein fed; feed efficiency ratio (FER) is the wet weight of fish per quantity of dry feed given. Survival in each tank was calculated at the end of the experiment.

Biochemical analysis

Proximate analyses of feed and tissue namely, moisture, crude protein (CP), ether extract, total carbohydrate and ash were carried out by standard method of AOAC (1995).

Enzyme assays

At the end of experiment all PL were killed. A 5% whole body homogenate in 250 ml of sucrose was immediately prepared. The homogenate was centrifuged at 5000 rpm for 20 min and the supernatant collected in a sample vial and kept frozen at −20°C until used. All enzyme assays were performed within 2 days. The aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were assayed as described by Wootton (1964). These enzyme activities were expressed as n mole of the product released per mg protein at 37°C. The product was oxaloacetate and sodium pyruvate for AST and ALT respectively. Protein quantification was carried out according to Lowry et al. (1951) using bovine serum albumin as the protein standard. All enzyme assays were the mean values of four readings.

Table 1
Estimated composition of the experimental diet (% dry weight)

Ingredients	T_0	T_1	T_2	T_3	T_4
Casein (fat free)[1]	35.12	35.12	35.12	23.86	13.11
Gelatin[2]	5.00	5.00	5.00	5.00	5.00
Dextrin[3]	10.00	10.00	10.00	10.00	10.00
Chitin[4]	–	5.00	10.00	–	–
SHM[5]	–	–	–	22.00	44.00
Starch[6]	30.00	30.00	30.00	30.00	18.89
Cellulose[7]	10.80	5.88	0.88	0.14	–
CMC[8]	1.00	1.00	1.00	1.00	1.00
Sunflower oil + codliver oil (2 : 1)	5.00	5.00	5.00	5.00	5.00
Vitamin + mineral mixture[9]	2.00	2.00	2.00	2.00	2.00
Vitamin C[10]	0.50	0.50	0.50	0.50	0.50
Vitamin B complex[11]	0.50	0.50	0.50	0.50	0.50

Butyl hydroxy toluene (BHT) added at 0.02% of the assessed oil; (1) casein fat-free: 75% CP (2) gelatin: 96% CP; (3) dextrin; (4) chitin; (5) shrimphead meal; (6) starch; (7) cellulose; (8) carboxy methyl cellulose (1–8 was supplied by Sd-Fine Chemicals Ltd); (9) composition of vitamin mineral mixture (Argimin) (quantity kg^{-1}): vitamin A, 6 25 000 IU; vitamin D_3, 62 500 IU; vitamin E, 250 mg; nicotinamide, 1 g; Cu, 312 mg; Co, 45 mg; Mg, 6 g; Fe, 1.5 g; Zn, 2.13 g; iodine, 156 mg; Se, 10 mg; Mn, 2 g; Ca, 247.34 g; P, 114.68 g; S, 12.2 g; Na, 5.8 mg; K, 48.05 mg; (10) Stay C (Hoffman La Roche, Nutley, NJ, USA); (11) Composition of vitamin B complex (quantity g^{-1}). Thiamine mononitrate, 20 mg; pyridoxin hydrochloride, 6 mg; vitamin B_{12}, 30 mg; niaciamide, 200 mg; Ca panthenate, 100 mg; folic acid, 3 mg; bioton, 200 mg.

Table 2
Proximate composition (g/100) of experimental diet (% dry matter basis ± SE)

		Purified chitin (%)		Natural chitin (%)	
	0	5	10	5	10
	T_0	T_1	T_2	T_3	T_4
Moisture	9.8	9.2	8.2	8.8	11.0
Protein	33.8	34.8	34.6	33.8	33.0
Ether extract	4.6	4.7	1.6	4.7	4.6
Organic matter	98.8	98.1	98.4	90.6	89.0
Total carbohydrate	55.3	58.7	59.1	54.4	54.4
Digestible energy	397.8	416.3	417.3	397.7	378.9
Ash	1.18	1.9	1.6	9.4	11.0

Mean values are derived from three replicates (n = 3).

Table 3
Biochemical composition of post-larvae *Macrobrachium rosenbergii* fed different experimental chitin content in the diets

Treatments	Moisture	Crude protein	Ether extract	Ash	Total carbohydrate
T_0	81.0 ± 0.6^{c}	47.5 ± 0.5^{c}	5.4 ± 0.2^{b}	6.2 ± 0.2^{b}	40.9 ± 0.6^{ab}
T_1	81.3 ± 0.1^{c}	49.4 ± 0.3^{b}	5.2 ± 0.2^{b}	7.2 ± 0.2^{a}	38.2 ± 0.6^{c}
T_2	84.1 ± 0.1^{a}	48.6 ± 0.4^{bc}	4.9 ± 0.1^{b}	5.3 ± 0.2^{c}	41.2 ± 0.3^{a}
T_3	80.0 ± 0.1^{d}	48.5 ± 0.6^{bc}	6.1 ± 0.1^{a}	6.4 ± 0.2^{b}	38.9 ± 0.7^{bc}
T_4	81.7 ± 0.2^{b}	51.6 ± 0.7^{a}	6.4 ± 0.2^{a}	6.6 ± 0.3^{ab}	35.4 ± 0.8^{d}

Values represent mean percentages dry weight ± SE. Mean values are data of three replicates where 15 individuals of each replicate have been pooled for analysis. Same superscripts in each column indicate no significant differences at $P > 0.05$ level. T_0–T_4 represent diet formulation as indicated in Tables 1 and 2.

Bacterial count of gut content

Bacterial plate count of the gut content was performed by preparing plates of overlaying tryptic soy agar (Sd-Fine Chemicals Ltd) with non-nutrient agar (Sd-Fine Chemicals Ltd) containing 1% (w/v) acid-precipitated colloidal chitin prepared according to West (1988). At the end of experiment, gut contents were removed from the PL, pooled and suspended in physiological saline under aseptic conditions and homogenized under ice at 2000 rpm for 1 min using a teflon-coated mechanical homogenizer. Serial dilution was made and six chitin overlay plates were inoculated by spread plate technique (0.1 ml of homogenate was added) from each treatment. The plates were incubated at 37°C for 48 h after which total and chitinolytic bacterial counts (indicated by a clearing halo) were made. Total plate count was performed by spread plate technique on nutrient agar plate. Serial dilution ranged from 10^{-3} to 10^{-6}. After incubation, isolated colonies were picked up and streaked at least three times in the nutrient agar medium to obtain purified colonies, which were maintained in nutrient agar. These purified colonies were again spotted in the colloidal chitin agar overlay plate to check the chitinolytic activity of bacteria and incubated for 48 h at 37°C.

Statistical analysis

Significant differences among treatment groups were tested by one-way ANOVA and comparison of any two mean values was by Duncan's multiple range test at 5% level of significance. The software programme SPSS (version 11) (SPSS Inc., Chicago, IL, USA) was used for statistical analysis.

Results

Biochemical composition of diet and experimental animals

Proximate composition of different experimental diets is given in Tables 1 and 2. The body compositions of *M. rosenbergii* PL at the end of experiment are given in Table 3. There were significant differences ($P < 0.05$) in the CP contents of experimental animals among different treatments. The highest CP content was recorded in the T_4 group. The ether extract was significantly higher ($P < 0.05$) in the T_3 and T_4 groups than in the other groups.

Growth parameters

The bodyweight gains of experimental groups recorded at 15-day intervals are shown in Fig. 1. Growth parameters, such as weight gain percentage, SGR, FCR, PER and survival (%), are given in Table 4. There were no significant differences ($P > 0.05$) in weight gain percentage, SGR, FCR and PER among T_1, T_3 and T_4 groups. Highest weight gain percentage

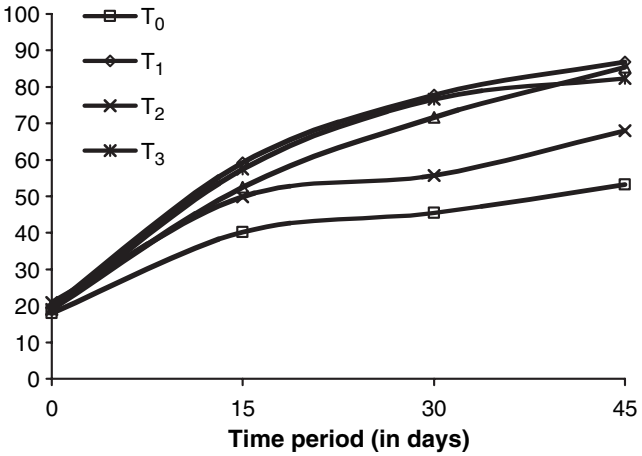

Fig. 1. Mean average body weight of post-larvae at 15-day intervals fed with different experimental diets. Mean values are data of three replicates where 15 individuals of each replicate were pooled for analysis. T_0–T_4 represent diet formulation as indicated in Tables 1 and 2

was found in the T_4 group (381.1 ± 30.9) and lowest in the T_0 group (195.4 ± 14.7). The same trend was found for SGR and PER. The FCR varied significantly ($P < 0.05$) among all treatments; the highest was in T_2 group (7.1 ± 0.3), the lowest in T_3 group (4.4 ± 0.4). There was no significant difference ($P > 0.05$) in FCR among T_1, T_3 and T_4 treatment groups. The mean survival rate of experimental groups varied significantly ($P < 0.01$). The highest survival was observed in T_3 group (80 ± 22.5) and lowest in T_2 group (40 ± 2.5). There was no significant difference ($P > 0.05$) in survival percentage among T_0, T_2 and T_4 groups.

Enzyme assays

There was no significant difference ($P > 0.05$) in AST activity among different treatments. ALT activity (Fig. 2) varied significantly among treatments. The highest and lowest ALT enzyme activity was found in T_2 and T_4 groups respectively. There was no significant difference among the T_0, T_3 and T_4 groups ($P > 0.05$), but these groups varied significantly from the T_1 and T_2 groups ($P < 0.05$).

Discussion

The dissolved oxygen level in the present study ranged from 7.68 to 8.40 ppm, which was within the normal range (7.80–9.00 ppm) for *M. rosenbergii* as reported by Subramanyam (1987). The observed temperature during the entire experiment was within the range of 29–30°C. Optimum temperature for *M. rosenbergii* was 28–31°C (Rodrigues et al., 1991; Valenti,

Table 4
Weight gain, SGR, FCR, FER, PER, and survival of post-larvae *Macrobrachium rosenbergii* fed different experimental chitin content in diets

Treatment	Weight gain percentage	SGR	FCR	PER	Survival
T_0	195.4 ± 14.7[b]	2.4 ± 0.1[b]	5.8 ± 0.3[b]	0.5 ± 0.02[b]	50.4 ± 7.5[b]
T_1	340.9 ± 18.0[a]	3.3 ± 0.1[a]	4.6 ± 0.6[c]	0.6 ± 0.05[a]	70.4 ± 5.6[a]
T_2	227.3 ± 17.9[b]	2.6 ± 0.1[b]	7.1 ± 0.3[a]	0.4 ± 0.01[b]	40.6 ± 3.0[b]
T_3	333.8 ± 16.7[a]	3.3 ± 0.1[a]	4.4 ± 0.4[c]	0.7 ± 0.04[a]	80.4 ± 5.7[a]
T_4	381.1 ± 30.9[a]	3.5 ± 0.1[a]	4.8 ± 0.6[c]	0.7 ± 0.06[a]	75.3 ± 5.7[a]

Values represent mean ± SE. Mean values are data of three replicates where 15 individuals of each replicate have been pooled for analysis. Same superscripts in each column indicate no significant differences at $P > 0.05$ level. T_0–T_4 represent diet formulation as indicated in Tables 1 and 2.

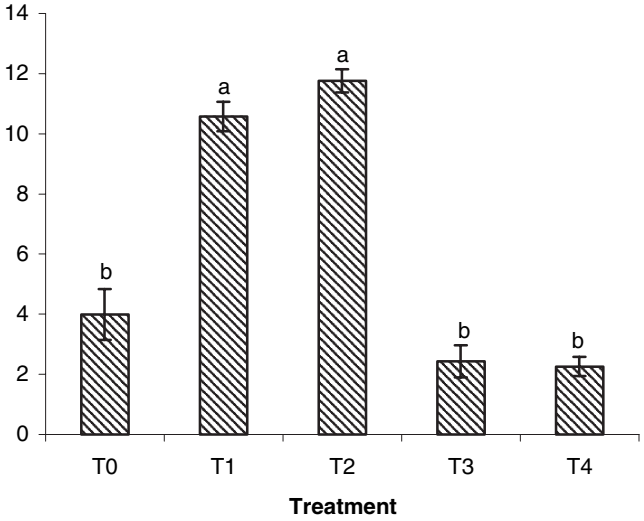

Fig. 2. Alanine amino transferase activity in post-larvae *Macrobrachium rosenbergii*. Mean values are data of three replicates where 15 individuals of each replicate were pooled for analysis. T_0–T_4 represent diet formulation as indicated in Tables 1 and 2

1996). The free carbon dioxide concentration was negligible due to low biomass, continuous aeration and routine water exchange. Water pH varied from 7.60 to 8.40, within the acceptable range (7.0–8.5) as suggested by New and Singhloka (1985). The ammonia nitrogen was below 0.50 ppm in all experiment tanks. For *M. rosenbergii* larval rearing, Valenti et al. (1998) recommended an ammonia nitrogen level below 0.50 ppm, which was almost the same in all experimental tanks because of isonitrogenous diet feeding in all groups. Nitrite nitrogen was below 0.01 ppm in all tanks. New (1990) recommended that nitrite levels should not be higher than 0.1 ppm.

The results of this study indicate that natural chitin dietary supplementation enhances *M. rosenbergii* growth. The groups fed diets containing the 5% purified chitin (T_1), 5% natural chitin or 22% shrimphead meal (SHM) (T_3) and 10% natural chitin or 44% SHM (T_4) showed higher weight gain (%), SGR, PER and lower FCR values than did the 10% purified chitin-fed group (T_2). This is in agreement with the findings of Shiau and Yu (1998) who reported a higher weight gain in shrimp (*P. monodon*) fed the 5% purified chitin diet than the 10% counterpart. The rate of nutrient absorption depends on the rate at which nutrients come into contact with the absorptive epithelium. Accordingly, the relative influence of dietary fibre on the movement of nutrients along the gastrointestinal tract will likely affect the nutrient absorption. Thus, the delay in stomach emptying caused by soluble dietary fibre might influence the nutrient absorption rate. Clark et al. (1993) reported that chitin digestibility in penaeid shrimp (*Litopenaeus vannamei*, *L. setiferus*, *Penaeus duorarum*) decreases as the purified chitin level increases in the diet. The trend of decreasing digestibility with increasing chitin level may be because of the limits to the enzyme hydrolysing capacity of penaeid shrimp. In the present study, it was found that *M. rosenbergii* PL fed with 10% purified chitin had a lower growth rate when compared with those fed 5% purified chitin. Excess chitin might act as a barrier for nutrient absorption. However, 10% chitin, which is equivalent to 44% shrimphead meal, did not show any growth depression. This might be due to the presence of some unidentified factor in shrimphead meal in the *M. rosenbergii* diet. Cocklin (1982) and Horst (1989) reported that arthropod chitin occurs naturally as a protein polysaccharide complex and additionally contains various lipid, pigments and minerals. Fox (1993) observed that increasing the level of dietary purified chitin did not significantly affect individual weight gain, SGR, FCR or survival ($P > 0.05$) of *P. monodon*. This explains why juvenile *P. monodon* could not utilize dietary purified chitin. Fox (1993) also reported that natural chitin (native chitin) such as that found in shrimphead meal might be of greater nutritional value to shrimp than the purified chitin. This may be because chitinase activity can be enhanced in the presence of protein, so that the chitinase assay using purified chitin may underestimate protein–chitin digestion (Lindsay, 1984; Danulat, 1986a,b). Purified chitin is prepared by using diluted mineral acids, which could remove a portion of these nutrients. It can therefore be concluded that shrimphead meal as a source of natural chitin might have a higher digestibility than the purified chitin. Lack of growth-enhancing effects of dietary purified chitin for *P. monodon* is probably a result of low digestibility rather than an inability to absorb or metabolize glucosamine (Kitabayashi et al., 1971; Fox, 1993).

With respect to AST activities, no significant differences among the various groups were observed ($P > 0.05$). This indicates that the rate of non-essential amino acids synthesis was similar in all groups and was not affected by the presence of purified or natural chitin. The amino acids produced might have been used for energy in high chitin-fed groups and might have been used for protein synthesis in lower chitin-fed groups. Purified chitin-fed groups had the higher ALT activity in comparison with natural chitin-fed groups and the control group, showing that animals were in stress when a higher percentage of purified chitin was used in the diet, which is in agreement with Vijayan et al. (1990) and Rao (1999).

The total viable bacteria count was gradually reduced as the chitin level increased, but the rate of reduction was less in natural chitin-fed groups in comparison with the purified chitin groups. This may be due to the antimicrobial properties of chitin (Gopakumar, 1997). An antimicrobial property was

more prominent in purified chitin than in the natural chitin. Several authors have postulated that gut bacteria are important for the production of chitin-digesting enzymes. The literature values for the total number of bacteria found in the digestive tracts of shrimp vary from 7.5×10^4 g^{-1} in *P. indicus* (Chandrmohan and Thomas, 1984) to 2.9×10^7 g^{-1} in *L. setiferus* (Hood and Meyers, 1973); of these, 67% showed chitinoclastic activity in the former study, whilst the proportion was 85% in *L. setiferus*. In the present study the total viable bacteria count decreased as the chitin level increased and no chitinoclastic bacteria were observed in any of the treatment groups, which is in agreement with the findings of Fox (1993), who reported restricted chitinoclasts and concluded that the synthesis of endogenous chitinase in the digestive gland of shrimp occurs at a slow rate and hence that juvenile shrimps are able to digest only small amounts of dietary chitin in the absence of bacterially produced chitinase.

It was clearly demonstrated in our study that incorporation of purified chitin enhanced the growth of *M. rosenbergii* PL. However, decreased growth at the 10% purified chitin supplementation level suggests that dietary chitin at this level depresses the growth rate of *M. rosenbergii* PL. Our study also showed that chitin incorporated into the diet of *M. rosenbergii* PL in either purified chitin or shrimphead meal will be directly utilized by the shrimp. In addition to protein, lipids and minerals associated with natural chitin (shrimphead meal) may be useful to the shrimp. Either 10% natural chitin, which is equivalent to 44% shrimphead meal or 5% purified chitin showed the best result among all groups. Moreover, 10% chitin via shrimphead meal would be more economical than 5% purified chitin because of its ubiquitous nature. Either the 10% chitin equivalent or the 44% shrimphead meal is recommended for promotion of *M. rosenbergii* PL growth.

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