

Original paper

THE PERFORMANCE OF LUPIN MEAL AS AN ALTERNATIVE TO FISHMEAL IN DIET OF JUVENILE *PENAEUS MONODON* UNDER POND CONDITIONS

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ABSTRACT

The present study was designed to investigate the performance of isonitrogenous practical diets containing different inclusion levels of dehulled lupin (*Lupinus angustifolius*) meal as an alternative for fish meal for juvenile *Penaeus monodon* (4.32 ± 0.57 g) reared in pens under pond conditions with a natural productivity. Five isonitrogenous experimental diets were formulated to contain 0, 10, 20, and 30% dehulled lupin (*L. angustifolius*) meal with a corresponding reduction in fish meal from 24 (0% replacement; D1 as control) to 18 (25% replacement; D2), 12 (50% replacement; D3) and 6% (75% replacement; D4) and a diet containing dehulled lupin (*L. albus*) meal formulated to be similar to D2 which was designated as D5 and used for comparison. Each treatment was tested in quadruplicate and arranged in a completely randomised design. In addition, a group of shrimp put in four pond pens was not fed, to estimate the contribution of pond natural production to shrimp growth.

There was no significant effect ($P > 0.05$) of different dietary treatments on mean individual weight gain (13.3–14.2 g), survival rate (88–93%) of shrimp, and feed conversion ratio (1.45–1.55). The presence of natural food in the pond was important to boost shrimp production and approximately 36–67% of growth of shrimp in the present study was supplied by natural food. Under such conditions, dehulled lupin (*L. angustifolius*) meal can replace up to 75% of protein from fish meal at inclusion levels of up to 30% in isonitrogenous practical diets without any adverse effects on growth, survival, and feed conversion ratio of juvenile *P. monodon*. Although this study was performed using a pond pen model on a small scale, the results obtained have provided useful information on the potential of lupin based meals in a commercial semi-intensive shrimp farming.

Key words: *Lupinus angustifolius*; *Lupinus albus*; fishmeal replacement; *Penaeus monodon*; pond culture

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INTRODUCTION

The performance of various isonitrogenous lupin based diets for juvenile *P. monodon* under laboratory conditions has been investigated and reported by Sudaryono *et*

al., 1999a, 1999b and 1999c. The results showed that lupin meal as a dietary source of protein can replace up to 75% of fish meal (Sudaryono *et al.*, 1999a) and up to 50% of soybean meal (Sudaryono *et al.*, 1999b) without any loss of the diet

performance. As reported by Sudaryono *et al.* (1999c), the performance of a diet containing lupin (*L. angustifolius*) meal was superior to that containing lupin (*L. albus*) meal. The findings under laboratory conditions suggest that variation in growth performance of shrimp fed with different lupin based diets was closely related to variation in the quality of dietary protein source, especially methionine and lysine content, feed intake, and dietary manganese content (Sudaryono *et al.*, 1999a, 1999b and 1999c), and pellet water stability and protein leaching (Sudaryono, 2000 and 2001). Whilst the growth results generated under laboratory conditions are more accurate in assessing the nutritional value of an artificial diet when this diet is the only source of required nutrients for shrimp, information obtained under laboratory conditions cannot be applied directly to the formulation of feeds for use within a semi-intensive pond-based farming system where the shrimp also derives a substantial part of their dietary nutrient needs from naturally available food organisms. Therefore, an investigation on the performance of diets containing lupin meal to replace fish meal on growth, survival and feed efficiency utilisation of juvenile *P. monodon* cultured under pond conditions was conducted. The results of this study are expected to be more applicable to commercial shrimp farms and consequently of more interest to farmers than those from experiments conducted in aquaria.

The present study was designed to investigate the performance of isonitrogenous practical diets containing different inclusion levels of dehulled lupin (*L. angustifolius*) meal as an alternative for fish meal for juvenile *P. monodon* reared in pens under pond conditions with a natural productivity.

MATERIALS AND METHODS

Diet Preparation

Five isonitrogenous experimental diets were formulated for this study. The four diets were formulated to contain 0, 10, 20, and 30% dehulled lupin (*L. angustifolius*) meal with a corresponding reduction in fish meal from 24 (0% replacement) to 18 (25% replacement), 12 (50% replacement) and 6% (75% replacement), respectively (Table 1). The diets were assigned as D1, D2, D3, and D4, respectively. The diet with no lupin meal (D1) was used as the control diet. One diet containing dehulled lupin (*L. albus*) meal formulated to be similar to D2 was designated as D5 and used for comparison. In the previous laboratory based study, use of this dehulled *L. albus* based diet formulation resulted in the best growth performance for juvenile *P. monodon* (Sudaryono *et al.*, 1999a). The use of this diet made it possible to compare the efficacy of *L. angustifolius* meal and *L. albus* meal in the diets for juvenile *P. monodon* reared in pens under pond conditions. The quantities of all other ingredients were kept constant except for the wheat flour and rice bran contents which were adjusted accordingly to keep all the diets isonitrogenous (approximately 40% crude protein). Diets were prepared according to procedures taken by Sudaryono (1998).

Feeding Trial Protocol

Juvenile shrimp with initial weight of 4.32 ± 0.57 g (mean \pm SD; N=240, range 0.7–1.2 g) was obtained from the commercial nursery ponds in Juwana, Central Java, Indonesia, where they had been cultivated for a month from PL₂₀. Prior to start of the experiments, shrimp was acclimatised to pond conditions and selected as an effort to compensate for variability in individual growth rate. Shrimp was stocked at the density of 10

animals/m²/pen. This stocking rate followed the previous nutrition studies on juvenile *P. monodon* reared in pond pens carried out by Pascual *et al.* (1990) and Cruz-Suarez *et al.* (1992). The animals were placed individually in the transportable compartments of each pen installed within a 1500 m² fertilised earthen pond. Each treatment in the experiment was tested in quadruplicate and arranged in a completely randomised design. In addition, a group of shrimp put in four pond pens was not fed, to estimate

the contribution of pond natural production to shrimp growth. Water quality conditions during the study were monitored using an Atago for dissolved oxygen and pH and hand refractometer for salinity.

To minimise handling stress for the 240 shrimp juveniles involved in the experiment, individual shrimp juveniles were not sexed prior to stocking. The experiment ran for 60 days and approximately one month was required to prepare for the experiment.

Table 1. Composition of the experimental diets (%)

Ingredient ¹	D1	D2	D3	D4	D5
Fish meal	24.0	18.0	12.0	6.0	18.0
<i>L. angustifolius</i> meal	–	10.0	20.0	30.0	–
<i>L. albus</i> meal	–	–	–	–	10.0
Defatted soybean meal	30.0	30.0	30.0	30.0	30.0
Squid meal	5.0	5.0	5.0	5.0	5.0
Shrimp meal	7.0	7.0	7.0	7.0	7.0
Wheat flour	20.0	20.0	20.0	16.0	20.0
Rice bran	8.0	4.0	–	–	4.0
Common ingredients ²	6.0	6.0	6.0	6.0	6.0

¹ Commercially available in Western Australia (see Chapter 3, Section 3.1); chemical and amino acid composition of each ingredient used in this study is shown in Appendices 3.1 and 3.2.

² Fish oil, 1.0; soy lecithin, 1.0; fish premix³, 1.0; carboxymethylcellulose (CMC), 2.0; and dicalcium phosphate, 1.0.

³ Fish premix contain: vitamin mix (mg/kg diet) (vitamin A, 10,000 IU; vitamin D, 1500 IU; vitamin E, 60; vitamin K, 1.5; niacin, 200; riboflavin, 37.5; calcium pantothenate, 125; vitamin B12, 20; thiamin, 15; pyridoxine, 15; folic acid, 5.5; biotin, 750; choline chloride, 100) and mineral mix (mg/kg diet) (cobalt, 0.2; iodine, 0.6; copper, 5.0; iron, 14.0; manganese, 55.0; zinc, 24.0).

Chemical and Data Analysis

All diets were analysed for dry matter, crude protein, crude lipid, crude fibre, ash, and gross energy contents following the standard methods of Association of Official Analytical Chemists (AOAC, 1990) (Table 2).

Diet performance was evaluated for mean weight gain (g), feed conversion ratio (FCR), and survival rate (%) by calculation according to the following equations (Allan *et al.*).

$$\text{Weight gain} = (W_{t_1} - W_{t_0})$$

Where W_{t_0} = mean body weight at day 0 (g); and W_{t_1} = mean body weight at day n (g)

$$\text{FCR} = \frac{\text{Dry feed consumed (g)}}{(W_{t_1} - W_{t_0})}$$

Survival rate was determined as percentage of number of shrimp surviving

at time n (Nt_n) to number of shrimp at time 0 (Nt_0) by the following equation:

$$\text{Survival rate (\%)} = (Nt_n / Nt_0) \times 100$$

All data were statistically analysed by using one-way analysis of variance (ANOVA) and multiple comparisons among treatment means were made with the Duncan multiple comparison test using the Statistical Analysis Software Program of SPSS (Release 6.1 for Windows). Prior to ANOVA, normality and homogeneity of variance of the data was assessed using the Kolmogorov-Smirnov test for Goodness of fit and the Levene test, respectively. Survival (%) data were subjected to an arc sin square root transformation prior to analysis. Covariance analyses were used to confirm that there were no effects of initial weights and sex ratio on growth parameters. Results were considered statistically significant at the level of $P < 0.05$ (Steel and Torrie, 1980).

RESULTS

Experimental Conditions

The water quality parameters (temperature, pH, salinity, dissolved oxygen (DO), and visibility) were recorded at 06.00 h, 12.00 h, and 18.00 h daily in six selected pens

within the experimental pond during the 60-day growout period. The average morning water temperature, pH, and DO was the lowest with the values of 29.7°C, 8.3, and 3.5 mg/L. The lowest average morning DO recorded was 1.7 mg/L on day 19 and the highest average DO values were 9.4 mg/L and 12.5 mg/L recorded at 18.00 h. The average water salinity values were relatively constant during the day ranging between 6 and 7 ppt. The average visibility was the highest in the morning with the value of 37 cm. During the experiment, water nitrite and ammonia-nitrogen levels measured weekly in six randomly selected pens within the pond were less than 1 mg/L.

Chemical Analyses

The results of chemical analyses of the experimental diets used in this experiment are shown in Table 2. The results showed that all diets had identical contents of crude protein (40.0–43.6%), crude fat (6.1–7.9%), crude fibre (4.0–4.5%), and gross energy (4052–4571 kcal/kg). Increase in replacement levels of fish meal with lupin meal resulted in the reduced dietary ash contents from 13.3 to 9.9%. Ash level in the diets based on *L. angustifolius* meal (D2) and on *L. albus* meal (D5) was similar (Table 2).

Table 2. Proximate composition of the test diets (on a dry weight basis)

Nutrient	D1	D2	D3	D4	D5
Dry matter (%)	96.9	96.7	95.4	95.2	96.0
Crude protein (%)	43.6	42.4	42.3	41.7	42.1
Crude fat (%)	7.0	6.9	6.1	6.3	7.1
Ash (%)	13.3	11.9	10.7	9.9	12.0
Crude fibre (%)	4.5	4.5	4.5	4.5	4.5
NFE ² (%)	31.6	34.3	36.4	37.6	34.3
Gross energy (kcal/kg)	4244	4401	4178	4258	4200

¹ Values are mean of pooled sample from three batches of diets

² Nitrogen free extract (calculated by difference)

Growout Studies

There was no significant effect of different dietary treatments on mean individual

weight gain of shrimp of different sexes. Females grew similarly to males in all treatments (average for all treatments: females 13.7 ± 0.8 g (range 10.7–17.8 g), males 13.1 ± 0.8 g (range 10.8–16.2 g)).

Average body weights of shrimp at 15-day intervals are presented in Figure 1 and summarised in Table 3. There was no significant difference in weight gain of shrimp fed with different experimental diets (D1, D2, D3, D4, D5) throughout the 60-day period in pond conditions. Weight gains achieved by shrimp after 15 days

were between 2.7–2.8 g, after 60 days - between 13.5–14.2 g. Weight gain achieved by the unfed shrimp was only 5.1 g at the conclusion of the experiment. Approximately 36–61% weight gains of shrimp were supplied by natural foods present in cages, within the pens within the pond, which can be shown by comparison of growth data obtained from the fed group and the unfed group throughout the experiment (see Table 3).

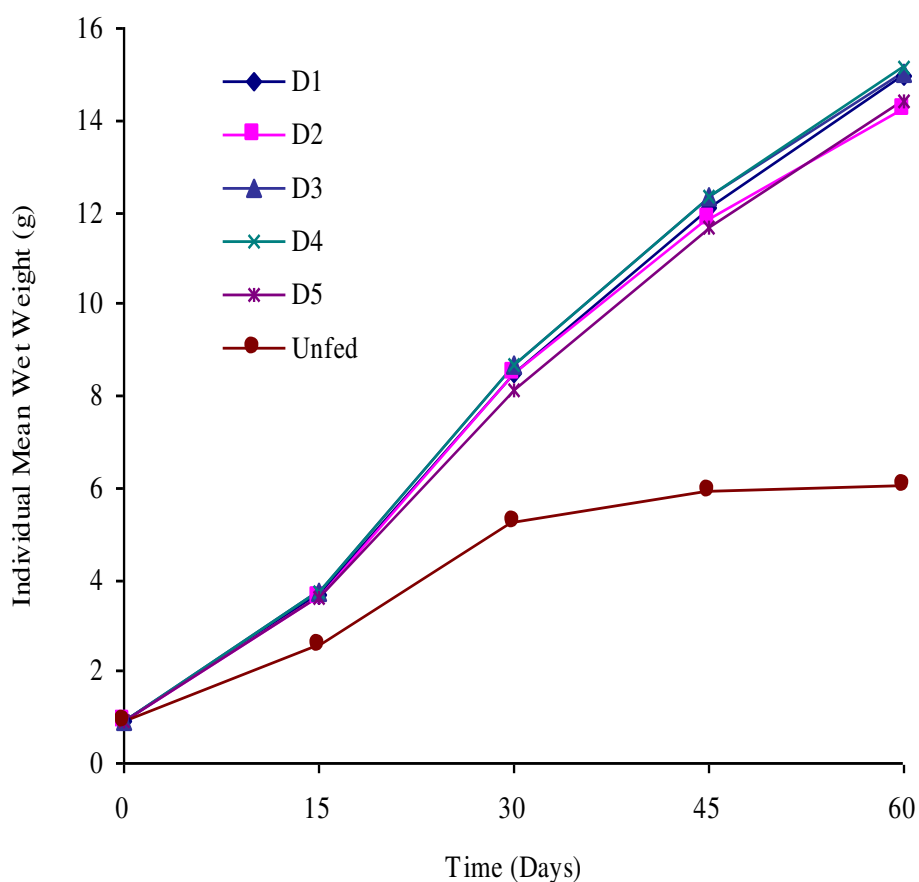


Fig. 1. Growth performance of juvenile *P. monodon* fed test diets in pens under pond conditions for the 60-day culture period.

FCR increased progressively with increase in the culture period from the range of 0.96–0.99 during the first 15 days to the range 1.45–1.55 during the last 15 days of the experiment (Table 4). Survival rates were high (>87%) and unaffected by different dietary treatments.

DISCUSSION

The low salinity in the study was related to a greater rainfall occurring during the period of the study, from February to

April. However, the low salinity in this experiment did not affect the growth rates of fed and unfed shrimp (see Table 5). This is in agreement with results of Venkataramiah *et al.* (1975), working with brown shrimp (*P. aztecus*) and Hysmith and Colura (1976), working with white shrimp (*P. setiferus*), who reported that growth of the shrimp cultured at 7–8 ppt and 15–17 ppt was comparable. Wickins (1976) and Fernandes and Achuthankutty (1997) reported that salinity had little or no influence on moulting frequency and growth.

Table 3. Individual growth, survival, and feed conversion ratio (FCR) of shrimp fed different experimental diets in pond pens for 60 days

Index	D1	D2	D3	D4	D5	Unfed
Initial weight (g)	0.89±0.09	0.92±0.14	0.94±0.12	0.91±0.14	0.89±0.12	0.90±0.12
Initial shrimp no.	40	40	40	40	40	40
Day 15						
Final weight (g)	3.64±0.42	3.60±0.48	3.74±0.59	3.74±0.66	3.61±0.37	2.57±0.25
Weight gain (g)	2.74±0.39	2.69±0.45	2.80±0.58	2.83±0.66	2.72±0.38	1.66±0.25
Growth rate (mg/day)	183 ± 26	179 ± 30	186 ± 38	189 ± 44	181 ± 25	111 ± 17
Final shrimp no.	38	37	40	39	37	37
Survival (%)	95.0±5.8	92.5±9.6	100 ± 0	97.5±5.0	92.5±5.0	92.5±9.6
FCR ²	0.97±0.15	0.99±0.17	0.97±0.19	0.96±0.21	0.98±0.14	–
Day 30						
Final weight (g)	8.48±0.95	8.50±0.73	8.65±0.95	8.68±1.13	8.12±0.62	5.28±0.67
Weight gain (g)	7.59±0.91	7.59±0.73	7.70±0.95	7.77±1.16	7.23±0.59	4.38±0.69
Growth rate (mg/day)	253 ± 30	253 ± 24	257 ± 32	259 ± 39	241 ± 20	146 ± 23
Final shrimp no.	38	37	40	39	37	36
Survival (%)	95.0±5.8	92.5±9.6	100 ± 0	97.5±5.0	92.5±5.0	90.0±14.1
FCR	0.87±0.11	0.86±0.08	0.86±0.11	0.86±0.12	0.90±0.08	–
Day 45						
Final weight (g)	12.1±1.4	11.8±1.0	12.4±1.3	12.3±1.5	11.7±1.0	5.93±0.81
Weight gain (g)	11.3±1.8	11.0±1.0	11.4±1.3	11.4±1.5	10.8±1.0	5.0 ± 0.8
Growth rate (mg/day)	250 ± 31	245 ± 24	254 ± 28	254 ± 34	240 ± 21	112 ± 18
Final shrimp no.	37	37	40	38	37	36
Survival (%)	92.5±5.0	92.5±9.6	100 ± 0	95.0±5.8	92.5±5.0	90.0±14.1
FCR	1.17±0.15	1.20±0.12	1.15±0.14	1.16±0.16	1.22±0.11	–
Day 60						
Final weight (g)	15.0±1.8	14.3±1.4	15.1±1.5	15.1±1.7	14.4±1.4	6.04±0.89
Weight gain (g)	14.1±1.7	13.3±1.4	14.1±1.5	14.2±1.8	13.5±1.5	5.1 ± 0.9
Growth rate (mg/day)	235 ± 29	222 ± 23	235 ± 25	237 ± 29	225 ± 24	86 ± 15
Final shrimp no.	37	35	38	35	35	36
Survival (%)	92.5±5.0	87.5±9.6	95.0 ± 10	87.5±5.0	87.5±5.0	90.0±14.1
FCR	1.47±0.20	1.55±0.16	1.45±0.15	1.45±0.18	1.52±0.18	–

¹ Values are means \pm SD of final shrimp number surviving and not significantly different ($P < 0.05$)

² FCR = total dry feed given (g)/total wet weight gain (g)

To compare growth in *P. monodon* reported in this study with the results reported by other authors, the data were expressed as growth rate in mg/day. Using this approach, the mean growth rates of 222–237 mg/day, achieved with diets tested in this experiments were comparable to or better than those previously reported by other workers for *P. monodon* juveniles under outdoor pond or cage-based farming systems. For example, Maguire *et al.* (1988) reported a 204 mg/day growth rate in 2.6 g shrimp fed a commercial Taiwanese feed (47% protein) and stocked at 15 animals/m² for 120 days, Chen *et al.* (1989) using 0.006 g shrimp fed a commercial feed for 120 days reported a growth rate of 228 mg/day, and Pascual *et al.* (1990) reported a growth rate of 206 mg/day by 2.8 g shrimp fed a practical diet (40% protein) and stocked at 10 animals/m² for 120 days. Allan and Maguire (1992a) noted a 160 mg/day growth by 3.5 g shrimp fed by a commercial Taiwanese feed (47% protein) and was stocked at 5 animals/m² for 59 days, Cruz-Suarez *et al.* (1992) reported a 175 mg/day increase in 2.2 g shrimp fed by a practical diet (49% protein) and was stocked at 10 animals/m² for 40 days, while Trino *et al.* (1992) reported a growth rate of 174 mg/day by 0.2 g shrimp fed by a practical diet with vitamin addition (35% protein) and was stocked at 5 animals/m² for 135 days. Allan *et al.* (1995) reported that shrimp averaging 4.5 g gained 169 mg/day fed by a commercial feed and was stocked at 15 animals/day for 71 days, while Trino and Sarroza (1995) reported a 183 mg/day increase by 0.006 g shrimp fed by a practical diet (42% protein) and was stocked at 7.5 animals/m² for 120 days and a 144 mg/day growth rate was reported by

Millamena and Trino (1997) using 0.2 g shrimp fed by a practical diet (35% protein) and was stocked at 10 animals/m² for 135 days.

Average individual growth rates of shrimp in the experiment were not significantly affected by any dietary treatment tested. This indicates that dehulled lupin (*L. angustifolius*) can be incorporated in the ration up to 30% total inclusion as an alternative for up to 75% of fish meal. No significant difference in growth was observed between shrimp fed with two diets formulated to contain the same inclusion levels of dehulled *L. angustifolius* meal and *L. albus* meal (D2 vs. D5) (Tables 1 and 4). Although the performance of these two different lupin species based diets (DAD vs. DND) was significantly different under laboratory conditions (Sudaryono *et al.*, 1999c), this difference did not appear when the diets were tested under pond conditions. It is evident that the lower protein quality, especially the deficiency in lysine and methionine or other essential nutrients in *L. albus* based diet, may have been compensated by natural food organisms available in the pond. These results are in agreement with those of other workers who reported that an inferior nutritional value of the diets tested for *P. monodon* under pond condition was compensated by natural food occurring in the ponds (Pascual *et al.*, 1990; Cruz-Suarez *et al.*, 1992; Trino *et al.*, 1992; Trino and Sarroza, 1995). All diets tested in the present study produced similar growth and survival results. This suggests that the presence of natural food within the pond can compensate for any important nutritional inadequacies amongst these test diets.

A deleterious effect of manganese, which was suggested in Sudaryono *et al.* (1999c) as a cause of the inferior performance of *L. albus* based diet compared with the performance of *L. angustifolius* in laboratory growout trials, did not appear when the diets were tested

under pond conditions. This finding indicates that the degree of sensitivity of juvenile *P. monodon* to the adverse effect of dietary manganese content probably varies with the health status of shrimp which is in turn related to ensuring adequate nutrient requirements of the animal.

The importance of natural food organisms as nutrient sources in the diet of penaeid shrimp has been stressed (Wyban *et al.*, 1987; Pascual *et al.*, 1990; Allan and Maguire, 1992a; Cruz-Suarez *et al.*, 1992; Trino *et al.*, 1992; Bombeo-Tuburan *et al.*, 1993; Allan *et al.*, 1995; Trino and Sarroza, 1995; Millamena and Trino, 1997). Anderson *et al.* (1987) estimated that approximately 53 to 77% of the growth of *P. vannamei* (initial weight 1.5 g), stocked at 20 shrimp/m², was due to grazing on pond biota and only 23 to 47% was supplied by added feed, while Garson *et al.* (1986) estimated that approximately 50% of the growth of *P. stylirostris* (initial weight 0.05 g), stocked at 5 shrimp/m², was contributed by natural food items present in the ponds. Gonzales (1988) estimated that contribution of natural pond biota to growth of juvenile *P. monodon* (initial weight 0.02 g/shrimp) was between 51 and 89%. In the present study, between 59 to 61%, 56 to 61%, 44 to 46%, and 36 to 38% of the growth of shrimp achieved at sampling days of 15, 30, 45, and 60, respectively were contributed by natural food organisms present in the pond (see Table 5). This finding indicates that the presence of natural food items in the fertilised pond is important in boosting shrimp production. However, the presence of natural food alone with no added artificial feeds (unfed group) cannot provide enough nutrients to increase the production of shrimp stocked at a density of 10 animals/m².

Pond preparation, as practised prior to the start of each experiment here, was effective in boosting unfed shrimp growth (control) during the first 30 days of

the experiment. However, growth in the unfed group declined thereafter. In other studies, such a decline has been related to a reduction in the abundance of natural food organisms present within ponds due to grazing by shrimp (Hanson and Goodwin, 1977; Rubright *et al.*, 1981; Maguire and Leedow, 1983; Wyban *et al.*, 1987; Gonzales, 1988; Allan and Maguire, 1992; Allan *et al.*, 1995). Although no measurement was performed to check the abundance of natural food items such as phytoplankton and meiofauna in these trials, the results obtained support this hypothesis. A decline in the abundance of natural food in the pond during the last 15 days of the experiments may also have been due to the fact that no regular fertilisation was applied during the course of the experiments. Fertilisation using urea and ammonium phosphate was applied only once during pond preparation and no organic fertiliser such as animal manure was applied. Under no artificial feed input, Wyban *et al.* (1987) reported that individual mean weekly *P. vannamei* growth was constant when about 36 kg of feedlot cattle manure per pond per week (1800 kg manure/ha/week) was applied regularly until the end of the experiment.

The low feed conversion ratio values, obtained in the present studies, could be partially explained by an improvement of the nutritive value of the diets due to the presence of natural food organisms in the ponds. The poorer FCR obtained during the last 15 days of both experiments may have resulted from a decline in the abundance of natural food. The placement of shrimp in the individual compartments may also have contributed to the low FCR values. Shrimp individually placed within a limited space could utilise feed more efficiently for growth rather than for other activities. No searching and competition for food among shrimp was likely to have occurred and this may also be responsible for the low FCRs obtained in the present study.

Apart from water quality, high survival rates of shrimp in this study could be attributed to the experimental design allowing no cannibalism to occur during the experiment due to placing shrimp individually in the compartments. Regular cleaning of the compartments and the pens of attached aquatic materials on every sampling day to maintain a good water circulation ensured sufficient DO and minimised the faecal material accumulation. These factors may also have accounted for the low mortality of shrimp observed during the study.

Overall, all lupin meal based diets tested under pond conditions exhibited similar performance. The presence of natural food in the pond was important to boost shrimp production and approximately 36–67% of growth of shrimp in the present study was supplied by natural food. Under such conditions, dehulled lupin (*L. angustifolius*) meal can replace up to 75% of protein from fish meal at inclusion levels of up to 30% in isonitrogenous practical diets without any adverse effects on growth, survival, and feed conversion ratio of juvenile *P. monodon*. Although this study was performed using a pond pen model on a small scale, the results obtained have provided useful information on the potential of lupin based meals in a commercial semi-intensive shrimp farming.

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