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PELLET WATER STABILITY STUDIES ON LUPIN MEAL BASED SHRIMP (*Penaeus monodon*) AQUACULTURE FEEDS : COMPARISON OF LUPIN MEAL WITH OTHER DIETARY PROTEIN SOURCES

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ABSTRACT

Nutritional quality of lupin based shrimp (Penaeus monodon) aquaculture feeds was evaluated in terms of pellet water stability. Two series of water stability experiments were carried out to study the effects of lupin meal inclusions as an dietary protein alternative for fish meal (Experiment 1) and soybean meal (Experiment 2) on percentage dry matter and protein leachings of the compounded test diets. Increasing the replacement levels of dietary fish meal with lupin meal resulted in significant decrease ($P<0.05$) of pellet water stability over the 480-minute immersion period. A significant improvement in water stability with the increase of the soybean meal replacement levels with lupin meal up to 50% was found, however, further increase above 50% substitution level resulted in reduced water stability of the diets. It is concluded that lupin meal can not totally replace either fish meal or soybean meal in shrimp formulated diets and a 50% replacement level of dietary fish meal or dietary soybean meal with lupin gives a promising good result in terms of pellet water stability for P. monodon diets.

I. INTRODUCTION

As discussed in a review of physical characteristics of feeds for shrimp (Sudaryono, 2000), the quality of a shrimp diet is determined not only by its nutritional value but also by its physical characteristics, especially water stability (Bengtson, 1993; Tacon, 1996). Studies of water stability of shrimp aquaculture feeds are especially important as shrimp are continuous and slow eaters (Cuzon *et al.*, 1982; Gadient and Schai, 1994; Lim and Cuzon, 1994). Therefore, ideally the diet should be water stable for a few hours so that no leaching of water-soluble nutrients prior to ingestion by shrimp occurs (Meyers and Zein-Eldin, 1972; New, 1976; Lovell, 1982; Lim and Persyn, 1989; Gadient and Schai, 1994; Tacon, 1991). Cuzon *et al.* (1994) suggested that crustacean diets should not lose more than 10% dry matter after 1 hour exposure in water. Lim and Cuzon (1994) in their review on water stability of shrimp diets concluded that composition of diets, feed manufacturing processes, and type and amount of binders used were major factors affecting water stability of pellets.

Currently, very little information is available on water stability of aquaculture diets containing lupin meal. Up to date, it has been reported that neither lupin particle size nor the amount of lupin meal substituting wheat flour up to 40% in the diets had any significant effect on water stability in terms of dry matter and protein leaching, although both factors affected the leaching of highly water-soluble components such as fructose (unpublished report of Australian Quality Ingredient Ltd., 1997). However, currently no information has been reported on pellet water stability of formulated shrimp diets containing

various lupin meal inclusions as a replacement for fish meal or soybean meal.

In the previous reports (Sudaryono *et al*., 1999a; 1999b; 1999c), the quality of various lupin meal based diets has been evaluated in terms of their nutritional value through studies of growth and digestibility for juvenile shrimp, *Penaeus monodon*. The study reported in this paper was designed to investigate the effect of lupin meal inclusion as an alternative for fish meal or soybean meal in a typical *P. monodon* aquaculture diet on pellet water stability in terms of dry matter and protein leaching.

II. MATERIALS AND METHODS

2.1. Experimental Protocol

Two series of pellet stability experiments were carried out for all *Penaeus monodon* formulated diets previously used in the growout and digestibility trials to evaluate the use of lupin meal (dehulled *Lupinus albus* seed) as an alternative for fish meal (Sudaryono *et al*., 1999a) or as an alternative for soybean meal (Sudaryono *et al*., 1999b). The first part of the experiment was designed to determine the degree of water stability of experimental diets expressed as the percentage of dry matter weight loss after immersion in seawater for different time periods. The second part of the experiment was designed to determine the protein leaching expressed as the percentage of protein loss on a dry matter basis after immersion in seawater for different time periods.

Two groups of five shrimp test diets containing different inclusion levels (0, 25, 50, 75, and 100%) of lupin meal instead of fish meal (Experiement 1) and soybean meal (Experiment 2). The test diets for Experiement 1 are designated as Diet 1, Diet 2, Diet 3, Diet 4 and Diet 5, respectively and the formulations of the test diets were shown in Table 1. While, the test diet for Experiment 2 are designated as Diet 6, Diet 7, Diet 8, Diet 9 and Diet 10, respectively and the diet formulations were shown in Table 2.

Table 1. Composition (g/100 g dry weight) of test diets (Sudaryono *et al*., 1999c)

Ingredient	% Replacement of fish meal with lupin meal				
	0 (Diet 1)	25 (Diet 2)	50 (Diet 3)	75 (Diet 4)	100 (Diet 5)
Fish meal	24.0	18.0	12.0	6.0	0
Lupin meal	0	10.0	20.0	30.0	40.0
Wheat flour	18.5	17.0	16.0	12.5	9.0
Rice bran	9.0	6.0	3.0	2.0	1.0
Common ingredient	48.5	49.0	49.0	49.5	50.0

Table 2. Composition (g/100 g dry weight) of test diets (Sudaryono *et al*., 1999b)

Ingredient	% Replacement of soybean meal with lupin meal				
	0 (Diet 6)	25 (Diet 7)	50 (Diet 8)	75 (Diet 9)	100 (Diet 10)
Soybean meal	30.0	22.5	15.0	7.5	0
Lupin meal	0	9.0	17.0	26.0	35.0
Wheat flour	18.5	20.0	22.5	23.0	22.5
Rice bran	9.0	6.0	3.0	1.0	0
Common ingredient	42.5	42.5	42.5	42.5	42.5

Triplicate samples of each diet (approximately 1 g) were placed in a small plastic cylinder (4 cm diameter, 6 cm tall) on a mesh base glued 2.5 cm from the bottom and arranged as a concave sieve. The design and construction of apparatus for the test followed the method developed by nutrition researchers of Bribie Island Aquaculture Research Centre, QDPI, Queensland, Australia. These cylinders were immersed and placed on the bottom of a cylinder plastic container (20 L) with seawater and aerated by air blower for certain periods. Seawater temperature was maintained at 28^{o}C. After immersing, each sample retained in the sieve was drained, redried at 105^{o}C overnight and reweighed for dry matter and protein determinations following the method of AOAC (1990). The dry matter and dry-weight based protein contents of the pellets before immersion were also determined by the same method. This technique is similar to that developed by Balazs (1973), Cruz-Ricque *et al.* (1987) and Maguire *et al.* (1988) for measuring the percentage dry matter loss of aquatic diets retained after immersion in water.

2.2. Determinations of Dry Matter and Protein Leachings

Percentage dry matter leaching of the test pellets was determined by the difference between the initial and final dry matter weights of pellets after immersion in seawater for predetermined certain periods 10, 20, 30, 40, 50, 60, 90, 120, 180, 240, 300, 360 or 480 minutes. Methods for determination and calculation of percentage dry matter are described in Section 3.6 of this chapter. Percentage dry matter leaching was calculated by the following equation:

% Dry matter leaching = $[(DMt_0 - DMt_n)/(DMt_0)\ g]\ x\ 100$

where: DMt_0 = weight of dry matter of the diet at t = 0 minute immersion; and

DMt_n = weight of dry matter of the diet after immersion at t = n minutes

Percentage protein leaching of the test pellets was determined by the difference between the initial and final protein contents of pellets after immersion in seawater for 30, 60, 120 or 240 minutes. The following equation was used to calculate the percentage protein leaching of experimental diets:

% Protein leaching = $[(PCt_0 - PCt_n)/(PCt_0)\ g] \times 100$

where: PCt_0 = protein content of the diet at t = 0 minute immersion;

PCt_n = protein content of the diet after immersion at t = n minutes

2.3. Data Analysis

All data were statistically analysed using one-way analysis of variance (ANOVA) and multiple comparisons among dietary treatment means were made with the LSD (Least Significant Difference) method 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. Pearson product correlation (2-tailed test) was used to detect any relationship between variables. Results were considered statistically significant at $P<0.05$ (Steel and Torrie, 1960).

III. RESULTS

3.1. Dry Matter Weight Loss

Results of each part of the experiments focussed on water stability (% dry matter weight loss) of various lupin meal based diets are summarised in Tables 3 and 4. Increasing the replacement levels of dietary fish meal with lupin meal

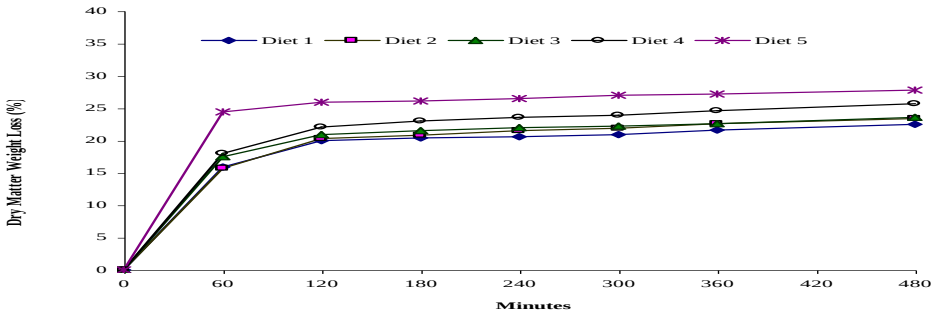
resulted in significant decrease of pellet water stability over the 480-minute (8-hour) immersion period. Increasing the exposure of the diet to seawater increased the percentage of dry matter weight loss from approximately 9-13% after 10 minutes to 22-28% after 480 minutes (Table 3). The dry matter weight loss dramatically increased within the first 120 minutes and did not increase much thereafter (Figure 1). The diet with the highest lupin meal inclusion level (100% replacement) consistently displayed the poorest water stability throughout the study.

Table 3. Dry matter weight loss (%) of experimental diets used in Experiment 1 (replacement of fish meal by lupin meal) exposed at different periods in seawater at 28°C. Values presented are mean and standard deviation (SD) of triplicate samples[1].

Period	% Replacement of Fish Meal by Lupin Meal				
	0 (Diet 1)	25 (Diet 2)	50 (Diet 3)	75 (Diet 4)	100 (Diet 5)
10 min	10.3 ± 1.0^{a}	10.3 ± 0.5^{a}	8.6 ± 2.3^{a}	10.9 ± 0.7^{a}	13.5 ± 1.9^{b}
20 min	12.9 ± 0.8^{a}	11.5 ± 0.8^{a}	12.6 ± 1.6^{a}	12.9 ± 0.3^{a}	15.9 ± 0.6^{b}
30 min	14.2 ± 0.5^{a}	13.8 ± 0.2^{a}	14.6 ± 0.2^{a}	15.6 ± 1.0^{b}	16.6 ± 0.2^{c}
40 min	14.3 ± 0.1^{a}	14.3 ± 0.3^{a}	15.8 ± 0.5^{b}	16.8 ± 0.4^{c}	20.5 ± 0.8^{d}
50 min	15.3 ± 0.4^{a}	15.1 ± 0.2^{a}	17.0 ± 0.3^{b}	17.3 ± 0.1^{b}	22.8 ± 0.1^{c}
60 min	15.9 ± 0.4^{a}	15.7 ± 0.4^{a}	17.5 ± 0.2^{b}	18.0 ± 0.2^{b}	24.4 ± 0.2^{c}
70 min	16.8 ± 0.2^{a}	16.9 ± 0.4^{a}	17.9 ± 0.3^{b}	18.4 ± 0.2^{c}	24.9 ± 0.2^{d}
80 min	16.9 ± 0.2^{a}	18.3 ± 0.2^{b}	18.5 ± 0.1^{b}	19.3 ± 0.5^{c}	25.2 ± 0.1^{d}
90 min	17.5 ± 0.2^{a}	18.9 ± 0.2^{b}	18.9 ± 0.3^{b}	20.5 ± 0.1^{c}	25.5 ± 0.1^{d}
120 min	20.0 ± 0.2^{a}	20.3 ± 0.3^{ab}	20.9 ± 0.3^{b}	22.1 ± 0.5^{c}	25.9 ± 0.1^{d}
180 min	20.4 ± 0.1^{a}	20.8 ± 0.2^{b}	21.5 ± 0.3^{c}	23.0 ± 0.2^{d}	26.1 ± 0.2^{e}
240 min	20.6 ± 0.2^{a}	21.5 ± 0.1^{b}	22.0 ± 0.1^{c}	23.6 ± 0.1^{d}	26.5 ± 0.2^{e}
300 min	20.9 ± 0.1^{a}	21.9 ± 0.5^{b}	22.2 ± 0.1^{b}	23.9 ± 0.4^{c}	27.0 ± 0.1^{d}
360 min	21.6 ± 0.2^{a}	22.6 ± 0.2^{b}	22.6 ± 0.3^{b}	24.6 ± 0.4^{c}	27.2 ± 0.1^{d}
480 min	22.5 ± 0.5^{a}	23.4 ± 0.4^{b}	23.6 ± 0.5^{b}	25.7 ± 0.3^{c}	27.8 ± 0.2^{d}

[1] Values in the same row having different superscripts are significantly different ($P<0.05$).

Figure 1. Water stability (expressed as % dry matter weight loss) of experimental diets used in Experiment 1 (replacement of fish meal by lupin meal) in different immersion times in seawater at 28°C.



Data on the percentage of dry matter weight loss of experimental diets used in Experiment 2 (replacement of soybean meal by lupin meal) are presented in Table 4. The results showed that there was a significant improvement in water stability with the increase of the replacement levels of soybean meal with lupin meal up to 50%. However, further increase above the 50% substitution level resulted in reduced water stability of the diets. The diet with 50% replacement containing a combination of 15% soybean meal and 17% lupin meal (see Table 2 for the dietary formulation) was the most water stable, while the diet with 100% replacement was the least stable (Table 4; Figure 2). The degree of dry matter weight loss of all diets dramatically increased from 6-12% after 10 minutes to 17-24% after 120 minutes and then was relatively constant between the immersion periods of 120 and 480 minutes.

Table 4. Dry matter weight loss (%) of experimental diets used in Experiment 2 (replacement of soybean meal by lupin meal) exposed at different periods in seawater at 28°C. Values presented are mean and standard deviation (SD) of triplicate samples[1].

Period	% Replacement of Soybean Meal by Lupin Meal				
	0 (Diet 6)	25 (Diet 7)	50 (Diet 8)	75 (Diet 9)	100 (Diet 10)
10 min	12.2 ± 0.6^{cd}	10.3 ± 1.0^{d}	6.1 ± 1.6^{a}	7.4 ± 0.4^{ab}	8.4 ± 2.0^{bc}
20 min	14.2 ± 0.1^{b}	12.9 ± 0.8^{ab}	10.9 ± 1.0^{a}	10.9 ± 1.5^{a}	14.4 ± 2.2^{b}
30 min	14.7 ± 0.1^{c}	14.2 ± 0.5^{bc}	12.5 ± 0.8^{a}	13.9 ± 0.5^{b}	17.4 ± 0.6^{d}
40 min	15.7 ± 0.2^{c}	14.3 ± 0.1^{ab}	13.9 ± 0.4^{a}	15.1 ± 1.0^{bc}	18.4 ± 0.9^{d}
50 min	16.5 ± 0.3^{b}	15.3 ± 0.4^{a}	14.7 ± 0.5^{a}	17.0 ± 0.4^{b}	19.7 ± 0.5^{c}
60 min	17.7 ± 0.2^{b}	15.9 ± 0.4^{a}	15.4 ± 0.2^{a}	17.7 ± 0.2^{b}	20.9 ± 0.3^{c}
70 min	17.9 ± 0.1^{c}	16.8 ± 0.2^{b}	16.4 ± 0.2^{a}	17.9 ± 0.1^{c}	21.6 ± 0.2^{d}
80 min	18.3 ± 0.2^{c}	16.9 ± 0.2^{a}	16.7 ± 0.1^{a}	18.0 ± 0.1^{b}	22.6 ± 0.4^{d}
90 min	18.5 ± 0.1^{d}	17.5 ± 0.2^{b}	16.9 ± 0.2^{a}	18.3 ± 0.2^{c}	23.6 ± 0.1^{e}
120 min	20.4 ± 0.1^{d}	20.0 ± 0.2^{c}	17.3 ± 0.1^{a}	18.5 ± 0.1^{b}	23.8 ± 0.1^{e}
180 min	21.2 ± 0.4^{d}	20.4 ± 0.1^{c}	17.8 ± 0.1^{a}	18.7 ± 0.2^{b}	23.9 ± 0.1^{e}
240 min	21.6 ± 0.1^{d}	20.6 ± 0.2^{c}	18.0 ± 0.1^{a}	18.9 ± 0.1^{b}	24.3 ± 0.2^{e}
300 min	22.1 ± 0.2^{d}	20.9 ± 0.1^{c}	18.3 ± 0.1^{a}	19.1 ± 0.1^{b}	24.8 ± 0.3^{e}
360 min	22.5 ± 0.1^{d}	21.6 ± 0.2^{c}	18.5 ± 0.1^{a}	19.3 ± 0.2^{b}	25.4 ± 0.2^{e}
480 min	23.5 ± 0.6^{b}	22.5 ± 0.5^{b}	20.3 ± 2.0^{a}	20.5 ± 0.4^{a}	25.9 ± 0.4^{c}

[1] Values in the same row having different superscripts are significantly different ($P<0.05$).

Figure 2. Water stability (expressed as % dry matter weight loss) of experimental diets used in Experiment 2 (replacement of soybean meal by lupin meal) in different immersion times in seawater at 28°C.

3.2. Protein Leaching

Loss (% on dry matter basis) of crude protein from various experimental diets after immersion in seawater at different periods of 30, 60, 120, and 240 minutes are summarised in Tables 5 and 6. Results of the study on the diets used for

Experiment 1 showed that an increase in replacement levels of dietary fish meal by lupin meal from 0% (diet 1) to 100% (diet 5) resulted in statistically significant increase of leaching levels of dietary protein after immersion in seawater for up to 120 minutes (Table 5). Within the 120-minute exposure time, protein stability of the diet with the highest lupin meal inclusion level, 40% (diet 5 with 100% replacement), was the poorest and the best protein stability was displayed by the diet with no lupin meal (diet 1, 0% replacement). After the first 30 minutes of immersion, 1.0–2.6% protein leaching was noted for the three lowest replacement diets (diets 1, 2, and 3) and 6.8–7.2% protein leaching for the two highest replacement diets (diets 4 and 5). However, after 120 minutes, no significant differences in percentages of protein leaching among the diets were observed (19.3–22.5%) (Table 5).

Table 5. Loss (% on dry weight basis) of crude protein of experimental diets containing various replacement levels of fish meal by lupin meal exposed to seawater at 28°C for different periods. Values presented are mean ± SD of triplicate samples[1].

Period	% Replacement of Fish Meal by Lupin Meal				
	0 (Diet 1)	25 (Diet 2)	50 (Diet 3)	75 (Diet 4)	100 (Diet 5)
30 min	2.6 ± 1.2^{a}	1.0 ± 0.7^{a}	2.0 ± 1.0^{a}	6.8 ± 1.8^{b}	7.2 ± 1.3^{b}
60 min	9.0 ± 0.7^{a}	9.4 ± 1.0^{a}	10.3 ± 1.4^{a}	11.7 ± 2.3^{a}	14.5 ± 1.6^{b}
120 min	12.9 ± 2.4^{a}	14.4 ± 1.5^{ab}	15.1 ± 0.7^{ab}	16.1 ± 0.7^{b}	16.1 ± 1.3^{b}
240 min	20.2 ± 3.8^{a}	20.0 ± 1.6^{a}	22.5 ± 1.7^{a}	19.3 ± 1.3^{a}	21.3 ± 1.6^{a}

[1] Values in the same row having different superscripts are significantly different ($P<0.05$).

For experimental diets used in Experiment 2, there was a significant difference in the degree of protein loss among the diets containing different replacement levels of soybean meal by lupin meal during the first 60 minutes in seawater. Within this period, the diets with lupin meal substituting 50% of soybean meal or more (diets 8, 9 and 10) tended to be more stable in terms of protein leaching than those containing the two lowest levels of lupin meal (diets 6 and 7) (Table 6). However, these differences disappeared when the diets were exposed to seawater for longer than 60 minutes with ranges of the protein leaching values from 12.4 to 13.2% and 19.2 to 23.1% by 120 and 240 minutes, respectively (Table 6).

Table 6. Loss (% on dry weight basis) of crude protein of experimental diets containing various replacement levels of soybean meal by lupin meal exposed to seawater at 28°C for different periods. Values presented are mean ± SD of triplicate samples[1].

Period	% Replacement of Soybean Meal by Lupin Meal				
	0 (Diet 6)	25 (Diet 7)	50 (Diet 8)	75 (Diet 9)	100 (Diet 10)
30 min	2.6 ± 1.2^{b}	1.3 ± 1.0^{ab}	0.9 ± 0.6^{a}	1.1 ± 0.9^{a}	0.5 ± 0.1^{a}
60 min	9.0 ± 0.7^{bc}	10.4 ± 1.2^{c}	8.8 ± 2.0^{bc}	6.4 ± 1.0^{a}	7.6 ± 1.2^{ab}

120 min	12.9 ± 2.4^a	13.2 ± 1.9^a	12.9 ± 1.3^a	12.4 ± 2.7^a	13.1 ± 1.5^a
240 min	20.2 ± 3.8^a	23.1 ± 1.1^a	21.8 ± 1.3^a	19.2 ± 0.6^a	21.1 ± 3.2^a

[1] Values in the same row having different superscripts are significantly different ($P<0.05$).

V. DISCUSSION

It is generally known that the physical quality of a pellet, especially its water stability, is affected by composition of the feed and the processing method employed. Addition of a binding agent may reduce the amount of residual fine particles and improve the water stability of the pellets. Since all experimental diets used in the present study were prepared by the same processing method and using the same amount and type of binder, difference in composition of the diets is the only factor influencing pellet water stability of the diets immersed in seawater for the same time period.

Pellet water stability was inversely related to the dietary level of lupin meal substituting fish meal (Table 3). The percentage dry matter loss increased significantly with increasing lupin meal prepared from dehulled *L. albus* seed in diets. This may be attributed to the low binding properties of lupin meal. Mohamed and Rayasduarte (1995) reported that lupin (*L. albus*) seed meal contains low levels of starch (3%). Low starch content of ingredients used may reduce binding strength of particles in the diets (Cuzon *et al.*, 1994; Lim and Cuzon, 1994).

Increase in the dietary fibre content with increasing lupin meal level in diets may be also responsible for the decreased water stability, which is in agreement with Bordner *et al.* (1986) and Akiyama *et al.* (1992) who reported that crustacean diets with high levels of fibre had reduced water stability. A similar result was also reported by Sudaryono *et al.* (1995) where a *P. monodon* diet containing 71% lupin meal exhibited the poorest water stability and this was associated with the higher fibre content (7.8%) of the diet compared with that of all other diets with no lupin meal (2.8–3.6%). Fox *et al.* (1994) and Penaflorida and Golez (1996) showed that the diets for *P. monodon* with high fibre content (7.1–8.4%) had significantly resulted in lower water stability than those containing low fibre content (5.1–6.4%). Diets with high dietary fibre content are relatively difficult to bind and this will result in fractures and cause a decrease in the water stability of the feed (Akiyama *et al.*, 1992). The physical quality of a shrimp feed usually improves with the lowering of the fibre content and total fibre level of commercial shrimp feeds should not exceed 4% (Akiyama and Dominy, 1991).

High fat level of feeds is also reported as one of the factors that can reduce water stability of feeds (Hastings, 1970; Lim and Cuzon, 1994). High dietary fat content may result in the reduced binding properties of pelleted feeds due to the overlubricating effect on compression in the die (Lim and Cuzon, 1994) and the lowering of starch gelatinisation during steam conditioning and the pelleting process (Hastings, 1970). Commercial shrimp feeds usually contain no more than 8% of total lipid (Akiyama and Dominy, 1991). An increase in crude fat content of the compounded diets used in Experiment 1 from 7.5% (diet 1) to 9.0% (diet 5) as reported by Sudaryono *et al*. (1999a) may be also one of the factors responsible for reduced water stability of diets. The increased fat content of the diets with

increasing dietary lupin meal inclusion rate was a reflection of a higher fat content of lupin meal compared with fish meal ingredient (Sudaryono *et al*., 1999a). This is in agreement with reports of Harris *et al.* (1986), Mohamed and Rayasduarte (1995), and Petterson *et al.* (1997) who stated that lupin meal generally contains higher fat levels than other cereal and pulse crops.

The poor water stability of diets 4 and 5 in Experiment 1 may be also attributed to lower wheat flour inclusion levels in the diets compared with those in the three other diets (9–12.5% vs. 16–18.5%; see Table 1). Wheat flour is rich in starch (>30%) which is the most common natural binder currently used in commercial shrimp feeds (Akiyama and Dominy, 1991). An increase in concentration of the raw starch in the diets with increasing dietary wheat flour levels may result in improved binding properties and water stability of the diets. Good binding properties of wheat flour in shrimp diets were also reported by Balazs *et al.* (1973), Lovell (1982), Shiau *et al.* (1991), and Akiyama *et al.* (1992).

Pellet water stability significantly differed among the diets containing various replacement levels of soybean meal with lupin meal (Table 4). Water stability of the 50% replacement diet (diet 8) was generally higher than that of diets with other replacement levels over the 480-minute exposure time in seawater. Probably, combination of two plant protein sources, soybean meal and lupin meal, at this replacement ratio results in optimal binding properties. Poor water stability of diets with high levels of dietary soybean meal (42% or higher) and of lupin meal (71%) was also observed by Lim and Dominy (1990) in *Penaeus vannamei* and by Sudaryono *et al.* (1995) in *P. monodon*, respectively. Synder and Kwon (1987) indicated that soybean meal is rich in structural, high molecular weight carbohydrates, such as cellulose, hemicellulose and pectins, but usually contains less than 1% starch. Lupin (*L. albus*) meal also has high fibre levels (Daveby and Aman, 1993) and low starch levels (approximately 3%) (Mohamed and Rayasduarte, 1995). Combination of all the raw starch present in wheat flour, soybean meal and lupin meal included at 22.5, 15, and 17%, res-pectively in diet 8 (50% replacement) might have resulted in the best gelatinisation (Table 2).

Among the five diets tested in Experiment 2, the diet with the highest lupin meal inclusion level of 35% (diet 10) consistently displayed the poorest water stability over the 480-minute immersion period in seawater (Table 4). The reduced water stability for this diet was likely to be due to higher fat content of the diet (9.5%) compared with the other four diets (Sudaryono et al., 1999b). This is similar to the result exhibited by diet 5 containing the highest lupin meal level of 40% used in Experiment 1 (Table 3). This effect was also shown by Cruz-Ricque *et al.* (1987) where percent dry matter loss of penaeid shrimp pellets significantly increased from 7.6 to 19% after one hour immersion in water with increasing dietary crude fat contents from 5.7 to 6.9%.

In general, the differing composition of experimental diets tested in the present study significantly influenced leaching rate of dietary protein in the first hour of immersion in seawater. An increase in exposure time period in seawater significantly increased percent protein leaching of the diets. However, no differences in the leaching rates of dietary protein were found among the diets used in Experiments 1 and 2 after immersion beyond this time up to 4 hours. The increased dietary protein leaching rates with increasing the exposure time period in seawater were also found by Bages and

Sloane (1981) in *P. monodon* diets, Lim (1993) in *P. vannamei* diets, and Mathias (1997) in black bream fish diets. They reported leaching rates ranging between 7–47% after 1–6 hours exposure. In the present study, the leaching rates of protein from all the diets tested at the maximum exposure time of 4 hours in seawater ranged between 11–25%.

Results of statistical analysis showed that in general there was no correlation between the percentage of dietary dry matter loss and protein leaching after immersion in seawater over 4 hours (240 minutes). This is in agreement with Goldblatt *et al.* (1980) who reported that no correlation was found between pellet water stability in water and nutrient leaching. This suggests that processes affecting dry matter water stability and protein leaching in pelleted diets are different.

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REFERENCES

Akiyama, D.M. and Dominy, W.G., 1991. Penaeid shrimp nutrition for the commercial feed industry. American Soybean Association and Oceanic Institute, Waimanalo, USA, 50 pp.

Akiyama, D.M., Dominy, W.G. and Lawrence, A.L., 1992. Penaeid shrimp nutrition. *In*: A.W. Fast and L.J. Lester (Eds.), Marine Shrimp Culture: Principles and Practices. Elsevier Science Publishers BV., pp. 535-568.

AOAC (Association of Official Analytical Chemists)., 1990. Official methods of analysis, 15 edition. Association of Official Analytical Chemists, Washington, D.C., 1094 pp.

Bages, M. and Sloane, L., 1981. Effects of dietary protein and starch levels on growth and survival of *Penaeus monodon* Fabricius postlarvae. Aquaculture, 25: 117-128.

Balazs, G.H., Ross, E. and Brooks, C.C., 1973. Preliminary experiments on the preparation and feeding of crustacean diets. Aquaculture, 2: 369-377.

Bengtson, D.A., 1993. A comprehensive program for the evaluation of artificial diets. Journal of the World Mariculture Society, 24: 285-293.

Bordner, C.E., D'Abramo, L.R., Conklin, D.E. and Baum, N.A., 1986. Development and evaluation of diets for crustacean aquaculture. Journal of the World Aquaculture Society, 17: 44-51.

Cruz-Ricque, L.E., Guillaume J. and Cuzon, G., 1987. Squid protein effect on growth of four penaeid shrimp. Journal of the World Aquaculture Society, 18: 209-217.

Cuzon, G., Guillaume, J. and Cahu, C., 1994. Review: composition, preparation and utilization of feeds for Crustacea. Aquaculture, 124: 253-267.

Cuzon, G., Hew, M., Cognie, D. and Soletchnik, P., 1982. Time lag effect of feeding on growth of juvenile shrimp, *Penaeus japonicus* Bate. Aquaculture, 29: 3-44.

Daveby, Y.D. and Aman, P., 1993. Chemical composition of certain dehulled seeds and their hulls with special reference to carbohydrates. Swedish Journal of Agricultural Research, 23: 133-139.

Fox, C.J., Blow, P., Brown, J.H. and Watson, I., 1994. The effect of various processing methods on the physical and biochemical properties of shrimp head meals and their utilization by juvenile *Penaeus monodon* Fabricius. Aquaculture, 122: 209-226.

Gadient, M. and Schai, E., 1994. Leaching of various vitamins from shrimp feed. Aquaculture, 124: 201-205.

Goldblatt, M.J., Conklin, D.E. and Brown, W.D., 1980. Nutrient leaching from coated crustacean rations. Aquaculture, 19: 383-388.

Gomes, E.F. and Kaushik, S.J., 1989. Incorporation of lupin seed meal, colzapro or triticale as protein/energy substitutes in rainbow trout diets. Proc. Third Int. Symp. Feeding and Nutrition in Fish, 28 August-1 September, Toba, Japan, pp. 315-324.

Hastings, W.H., 1970. Study of pelleted fish food stability in water. *In* : J.L. Gaudet (Ed.), Report of the 1970 Workshop on Fish Feed Technology and Nutrition, FAO/EIFAC and USID/BSFW, U.S. Government Printing Office, Washington D.C., pp. 75-80.

Harris, D.J., Spadek, Z.E. and Baseden, S., 1986. Chemical Evaluation of Lupin Seed as a Feed Grain. Agricultural Chemistry Laboratory, Feb. 1986, Western Australia, 42 pp.

Jasper, R. and Hoxey, M.**,** 1997. The effect of processed lupinseed on pellet hardness, water stability and leaching characteristics of prepared feeds for aquaculture. Australian Quality Ingredients Ltd., Western Australia, 13 pp. (unpublished paper).

Lim, C., 1993. Effect of dietary pH on amino acid utilization by shrimp (*Penaeus vannamei*). Aquaculture, 114: 293-303.

Lim, C and Cuzon, G., 1994. Water stability of shrimp pellets: a review. Asian Fisheries Science, 7: 115-127.

Lim, C. and Dominy, W.G., 1990. Evaluation of soybean meal as a replacement for marine animal protein in diets for shrimp (*Penaeus vannamei*). Aquaculture, 87: 53-63.

Lim, C. and Persyn, A., 1989. Practical feeding - penaeid shrimps. In: T. Lovell (Editor), Nutrition and Feeding of Fish. Van Nostrand Reinhold, New York, pp. 205-222.

Lovell, T., 1982. Status of penaeid shrimp nutrition and feed practices (Fish feed & Nutrition, part 2). Aquaculture Megazine, Sept.-October, 44 pp.

Maguire, G.B., Allan, G.L., Baigent, R. and Frances, J., 1988. Evaluation of the suitability of some Australian and Taiwanese diets fed to leader prawns (*Penaeus monodon*) in ponds. *In*: L.H. Evans (Ed.), Proceedings: First Australian Shellfish Aquaculture Conference. Curtin University of Technology, Perth, pp. 89-104.

Mathias, J., 1997. The effect of varying levels of dietary protein on protein digestibility in juvenile black bream (*Acanthopagrus butcheri*). Unpublished Honours Thesis. Curtin University of Technology, 66 pp.

Meyers, S.P. and Zein-Eldin, Z.P., 1972. Binders and pellet stability in development of crustacean diets. Proceedings of the World Mariculture Society, 3 : 351-364.

Mohamed, A.A. and Rayasduarte, P., 1995. Composition of *Lupinus albus*. Cereal Chemistry, 72: 643-647.

New, M.B., 1976. A review of dietary studies with shrimp and prawns. Aquaculture, 9: 101-144.

Penaflorida, V.D. and Golez, N.V., 1996. Use of seaweed meals from *Kappaphycus alvarezii* and *Gracilaria heteroclada* as binders in diets for juvenile shrimp *Penaeus monodon*. Aquaculture, 143: 393-401.

Petterson, D.S., Sipsas, S., Mackintosh, J.B., 1997. The Chemical Composition and Nutritive Value of Australian Pulses (2nd Edition). Grains research and Development Corporation, Canberra, 65 pp.

Shiau, S.Y., Kwok, C.C. and Chou, B.S., 1991. Optimal dietary protein level of *Penaeus monodon* reared in seawater and brackishwater. Journal of the Japanese Society of Scientific Fisheries, 57: 711-716.

Steel, R.G.D. and Torrie, J.H., 1960. Principles and Procedures of Statistics. A Biometrical Approach. McGraw Hill, New York, 481 pp.

Sudaryono, A., 2000. Experimental assessment of shrimp aquaculture feeds: a review of physical characteristics of feeds and growout studies. Aquaculture Indonesia, 1 (3): 163-171.

Sudaryono, A., Hoxey, M.J., Kailis, S. G. and Evans, L. H., 1995. Investigation of alternative protein sources in practical diets for juvenile shrimp *Penaeus monodon*. Aquaculture, 134: 313-323.

Sudaryono, A., Tsvetnenko, E. and Evans, L.H., 1999a. Evaluation of potential of lupin meal as an alternative to fish meal in juvenile *Penaeus monodon* diets. Aquaculture Nutrition, 5: 277-285.

Sudaryono, A., Tsvetnenko, E. and Evans, L.H., 1999b. Replacement of soybean meal by lupin meal in practical diets for juvenile *Penaeus monodon*. Journal of the World Aquaculture Society, 30: 46-57.

Sudaryono, A., Tsvetnenko, E., Hutabarat, J., Supriharyono and Evans, L.H., 1999c. Lupin ingredients in shrimp (*Penaeus monodon*) diets: influence of lupin species and

types of melas. Aquaculture, 171: 121-133..

Synder, H.E. and Kwon, T.W., 1987. Soybean utilization. Van Nostrand Reinhold, New York, NY, 346 pp.

Tacon, A.G.J., 1991. Vitamin nutrition in shrimp and fish. In: D.M. Akiyama and R.K.H. Tan (Editors), Proceedings of the Aquaculture Processing and Nutrition Workshop, Thailand and Indonesia, September 19-25, 1991. American Soybean Association, Singapore, pp. 10-41.

Tacon, A.G.J., 1996. Nutritional studies in crustaceans and the problems of applying research findings to practical farming systems. Aquaculture Nutrition, 1: 165-174.