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Digestibility and Pellet Water Stability Studies on Azolla Meal–Based Diets for Juvenile Black Tiger Shrimp, *Penaeus monodon*

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Abstract

Agung Sudaryono. 2006. Digestibility and pellet water stability studies on azolla meal–based diets for juvenile black tiger shrimp, *Penaeus monodon. Aquacultua Indonesiana*, 7(1) : 19–27. The apparent digestibility coefficients of dry matter (ADMD) and crude protein (APD) and water stability in five isonitrogenous practical diets (40% protein) containing azolla (*Azolla pinnata*) meal as a replacement for soybean meal were determined with juvenile *Penaeus monodon* (initial body weight, 0.49±0.03 g) through digestibility and pellet water stability trials. An indirect method using chromic oxide (Cr_2O_3) as an indicator was employed for the determination of digestibility. The water stability experiment was carried out to study the effects of azolla meal (AM) inclusions as a dietary protein alternative to soybean meal (SBM) on percentage dry matter leaching of the tested diets. Increasing replacement levels of azolla meal with soybean meal as protein source in the diets (0, 25, 50, 75, and 100%) did not significantly affect (P>0.05) on apparent dry matter digestibility (ADMD) and apparent protein digestibility (APD) with a range of 76.2–77.0% and 79.7–82.0%, respectively. No significant differences (P>0.05) in pellet water stability during the first 3 hours immersion for all diets (<9.8% feed loss) were also found in the study. However the highest inclusion level of dietary azolla meal (100% replacement) showed a worse water stability than the other diets after 3 hours immersion period. The results indicate that azolla meal–based diets could be digested as efficiently as soybean meal–based diets by juvenile *P. monodon* and have potential as an alternative plant protein source in shrimp diets.

Keywords: Digestibility; Water stability; Azolla meal; Practical diet; *Penaeus monodon*

Abstrak

Koefisien daya cerna bahan kering (ADMD) dan daya cerna protein (APD) pakan serta kestabilan pakan dalam air dari 5 pakan uji berkadar protein sama (40%) yang mengandung tepung azolla (*Azolla pinnata*) sebagai pengganti tepung kedelai ditentukan dengan memakai hewan uji juvenile udang windu *Penaeus monodon* (berat bobot awal rataan, 0.49±0.03 g) melalui serangkaian eksperiemen daya cerna dan kestabilan pakan. Metode tidak langsung dengan bahan cromium oksida (Cr_2O_3) sebagai indicator digunakan untuk penentuan studi daya cerna. Uji kestabilan pakan dilakukan untuk mengetahui pengaruh penggantian konsentrasi tepung kedelai secara bertahap dengan tepung azolla dalam pakan terhadap kelarutan bahan kering pakan uji. hasil menunjukkan bahwa peningkatan konsentrasi penggantian sumber protein dari tepung kedelai dengan tepung azolla dari 0, 25, 50, 75, and 100% tidak berpengaruh nyata (P>0.05) terhadap koefisien daya cerna bahan kering pakan (ADMD, 76.2–77.0%) dan koefisien daya cerna protein pakan (APD, 79.7–82.0%). Demikian juga tidak ada perbedaan nyata ditemukan (P>0.05) antar kelompok pakan uji pada uji kestabilan pakan selama 3 jam pertama dengan daya larut pakan dalam air <9.8%. Namun ditemukan bahwa pada konsentrasi penggantian 100% tepung kedelai dengan tepung azolla dalam pakan memberikan pengaruh kestabilan pakan yang paling rendah bila dibandingkan dengan pakan uji yang lain setelah pengujian dengan perendaman pakan dalam air selama lebih dari 3 jam. Hasil studi mengindikasikan bahwa pakan dengan tepung azolla sebagai sumber protein dapat dicerna sama efisiennya dengan pakan yang mengandung tepung kedelai oleh juvenile udang windu. Hasil studi ini juga menunjukkan bahwa tepung azolla adalah sebagai salah satu sumber protein nabati yang potensial menggantikan tepung kedelai dalam pakan udang.

Kata kunci: Daya cerna; Kestabilan pakan; Tepung azolla; Pakan udang; *Penaeus monodon*

Introduction

Feeding trials and chemical analysis are the starting point in crustacean nutrition studies. However digestibility studies are believed to be an effective biological approach to assessing the nutritional value of aquaculture feeds (Lee and Lawrence, 1985; Akiyama *et al*., 1989; Sudaryono and Ambariyanto, 1999; Thiessen *et al*., 2003; Wu *et al*., 2006). A formulated diet may appear from the chemical composition to be an excellent source of nutrients but will be of little value unless it can be digested and absorbed. Moreover, the quality of a shrimp diet is determined not only by its nutritional value but also by its physical characteristics, especially water stability (Bengston, 1993; Tacon, 1996). So that the studies of digestibility and water stability to evaluate the quality of a shrimp diet is essentially required.

Apparent digestibility coefficients of dry matter and protein in formulated diets for crustacean diets have been determined by an indirect method using the difference in ratios of ingested and egested marker (Cr_2O_3) and nutrient (Catacutan, 1991; Shiu and Peng, 1992; Clark *et al.*, 1993; Sudaryono *et al*., 1996; Brunson *et al*., 1997; Sudaryono and Ambariyanto, 1999). Feedstuff substitution procedures described by Cho *et al.* (1982) and refined by Forster (1999) enable the apparent digestibility of a single ingredient in a multiingredient diet to be determined. To date, however, apparent digestibility coefficients of practical diets containing azolla (*Azolla pinnata*) meal as an alternative protein source to soyben meal for juvenile *Penaeus monodon* are unavailable. Due to the nutritional value of a feed depends on the extent to which it is utilized by a particular species, digestibility coefficients for feeds or ingredients would be valuable in formulating efficient diets.

Studies of water stability of shrimp aquaculture feeds are especially important as shrimp are continous 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 (Lovell, 1982; Lim and Persyn, 1989; Tacon, 1991). Crustacean diets should not lose more than 10% dry matter after an hour exposure in water (Cuzon *et al*., 1994). It has been reported by Sudaryono (2001) that inclusion levels above 50% of lupin meal to replace protein sources of fish meal and soybean meal in practical diets resulted in reduced water stability of the diets. However, currently no information is available on pellet water stability of formulated juvenile *P. monodon* diets containing various azolla meal inclusions as a replacement for soybean meal.

The aim of this study was to evaluate the performance of *P. monodon* practical diets formulated containing different replacement levels of azolla meal with soybean meal as plant protein source in terms of dry matter and protein digestibility coefficients and water stability.

Materials and Methods

Diet Preparation

All test feed ingredients (except for azolla meal) were collected from commercial sources. Azolla meal was prepared in the laboratory from a aquatic plant of *Azolla pinnata*. The fresh azolla leafs were dried, grounded and passing them through a 500 µm screen. Five practical diets were formulated to contain 40% crude protein. Experimental diet formulations are presented in Table 1. Chromic oxide (Cr_2O_3) was added to the diets at an inclusion rate of 0.5% as the digestibility marker. All dry feed ingredients were mixed in a commercial food mixer for 15 minutes, where after oil was gradually added while mixing constantly. Thirty milliliters of water was slowly blended into the mixture for each 100 g of the diet. The diets were produced in a noodle–like shape of 2.0 mm in diameter using a meat mincer. Then the pelleted diets were dried overnight at 55°C, packed in plastic bags and stored in a freezer until used.

Digestibility Trial

Juvenile *P. monodon* were obtained from a commercial shrimp hatchery and acclimated to laboratory conditions for a week in two–500–L circular fiberglass tanks. During this period, shrimp received a commercial feed. After the first acclimation period in the laboratory, 30 shrimp with an initial mean weight of 0.49±0.03 g were randomly assigned to each of the five test diets and acclimated to the digestibility tank equipped with a flow–water system (salinity 33 ppt). Fifteen 75–L rectangular blue plastic tanks each housed ten

shrimp with three tanks replicates per treatment. Water quality parameters in the tanks were monitored routinely using a digital temperature for temperature, an ATAGO 0–100 g/L hand refractometer for salinity, a Cyberscan 100 electric pH meter for pH, and a Jenway 9071 DO meter for dissolved oxygen, respectively. These included temperature, salinity, pH, and dissolved oxygen which ranged from 28–32°C, 25–28 ppt, 8.0–8.5, and 6.2–7.0 mg/L, respectively.

During the preliminary period, shrimp were adapted to consume each experimental diet with a daily feeding rate of 5% body weight given twice per day. This adaptation period was required to stabilize diet intake and digestibility with the new diet (Sarac *et al.*, 1991). During the collection period of 3 weeks, shrimp were fed twice daily in the morning at 09.00 and in the afternoon at 15.00. Faecal material was collected 5 h after feeding by siphoning the bottom of the tank and separated from uneaten feed by sieving onto a fine mesh screen and hand sorting. Faeces were rinsed very gently with destilled water for a few seconds to separate other materials (e.g. dirt particles). This rinsing was not observed to cause fragmentation and loss of faeces. The faeces were then pooled and stored at –20°C. After termination of the collection period, frozen faecal material was freeze–dried and the dried samples were ground, thoroughly mixed, and kept frozen at –20°C until analysis.

Dry matter and crude protein of diet and faecal samples were determined following the standard methods of AOAC (1990) using an oven and a Tecator Digestion System 20 1015 and Tecator Kjeltec 1030 Auto Analyser, respectively. Chromic oxide content of all experimental diets and faecal samples was analysed according to the method of Williams *et al*. (1962) using atomic absorption spectrophotometry (AAS) after dry ashing and digesting with phosphoric acid–manganese sulphate solution and potassium bromate solution. All chemical determinations are reported on a dry matter basis.

Water Stability Trial

Triplicate samples of each formulated 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, Queensland Department of Primary Industries, Australia (unpublished document, 1993). These cylinders were immersed on the bottom of a cylinder black plastic container (20 L) with seawater and aerated by air blower for certain periods. After immersing, each sample retained in the sieve was drained, redried at 105°C overnight using an oven and reweighed for dry matter. The dry matter content of the pellets before immersion in the water was also determined by the same method (AOAC, 1990). This technique is similar to that developd by Balazs *et al*. (1973), Cruz–Ricque *et al*. (1987), Maguire *et al*. (1988), and Sudaryono (1998).

Data Analysis

The apparent digestibility coefficients of dry matter (ADMD, %) and crude protein (APD, %) were calculated by the equation as follows:

Digestibility (%) = 100 – 100 [(Cd/Cf) x (Nf/Nd)]

where:
Cd = % chromic oxide in diet;
Cf = % chromic oxide in faeces
Nf = % nutrient in faeces; Nd = % nutrient in diet

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 (33 ppt) for certain predetermined periods of 30, 60, 120, 180, 240 or 480 minutes. Percentage dry matter leaching was calculated by the following equation:

% Leaching = [(DMt0 – DMt*n*) / (DMt0) g] x 100

where:
DMt0 = weight of the diet dry matter at t=0 minute immersion
DMt*n* = weight of the diet dry matter after immersion at t=*n* minutes

A completely randomized design without sub–sampling was used with each tank as the experimental unit and the observed parameter values were tank means. All data were statistically analysed 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 SPPS for Windows. Results were considered statistically significant at the level of $P<0.05$.

Results

The coefficients of ADMD and APD of all five treatment diets are presented in Table 2. All diets and faeces had similar chromic oxide, dry matter and protein contents. The ADMD and APD of experimental diets ranged from 76.2 to 77.0% and 79.7 to 82.0%, respectively. No significant differences ($P>0.05$) in ADMD or APD coefficients were found among five experimental diets, indicating that digestibility of the diets was not influenced by increase in the replacement levels of dietary soybean meal with azolla meal.

Results of the experiments focused on water stability (% dry matter weight loss) of various replacement levels of dietary soybean meal protein with azolla meal protein are summarized in Table 3. The results showed that there was not a significant improvement ($P>0.05$) in water stability with the any increase of the replacement levels of soybean meal with azolla meal up to 100% for immersion periods up to 120 minutes. However, further increase above 120 minutes (e.g. 180 to 240 min) immersion periods resulted in reduced water stability of the diets. Further more, on the immersion period of 480 minutes, the diets containing no azolla meal (RSA 0) and no soybean meal (RSA 100) had a similar worse water stability than other diets containing soybean meal and azolla meal mixtures (RSA 25, RSA 50, RSA 75).

Discussion

The nutrients leaching problem from the feed in shrimp nutrition studies involving digestibility determinations using radiolabelled chromic oxide as a digestibility marker has been discussed by Fenucci *et al*. (1982), Taechanuruk and Stickney (1982), and Clark *et al*. (1993). Excessive leaching of nutrients from feed or faeces can lead to an overestimation of digestibility coefficients. However, experiments carried out by Fennuci *et al*. (1982), Smith *et al*. (1985), Law *et al*. (1990), and Sudaryono (1998) provide evidence that there are no significant errors in the determination of feed digestibility coefficients for crustaceans due to nutrient and chromic oxide loss from the faeces provided immersion in seawater is less than 6 hours.

Table 1. Composition of experimental diets (% as fed basis)

Ingredient	Replacement levels of protein source of soybean meal with azolla meal				
	0% (RSA 0)	25% (RSA 25)	50% (RSA 50)	75% (RSA 75)	100% (RSA 100)
Defatted soybean meal	300	225	150	75	0
Azolla meal	0	120	240	360	480
Fish meal	230	230	230	230	230
Squid meal	50	50	50	50	50
Krill meal	50	50	50	50	50
Pollack liver oil	40	30	20	10	0
Cholesterol	10	10	10	10	10
Soybean lecithin	10	10	10	10	10
α–Starch	180	160	140	120	100
Vitamin mix	20	20	20	20	20
Mineral mix	20	20	20	20	20
Cr_2O_3	5	5	5	5	5
Carboxymethylcellulose	25	25	25	25	25
Filler (a–cellulose)	60	45	30	15	0
Proximate analysis (% dry matter basis)					
Moisture	7.81	5.54	6.28	6.32	6.80
Ash	9.78	12.36	14.23	17.60	20.07
Crude lipid	9.75	9.40	9.28	9.57	9.72
Crude protein	41.72	40.44	42.16	41.12	41.34

Table 2. Results of digestibility trial using juvenile *P. monodon* for the 42–day feeding period (mean + SD)[1]

Parameter	RSA 0	RSA 25	RSA 50	RSA 75	RSA 100
SBM : AZM (%)[2]	100 : 0	75 : 25	50 : 50	25 : 75	0 : 100
Cr_2O_3 (%)					
Diets	0.46 ± 0.03 [a]	0.45 ± 0.05 [a]	0.49 ± 0.02 [a]	0.47 ± 0.03 [a]	0.43 ± 0.04 [a]
Faeces	1.92 ± 0.27 [a]	1.86 ± 0.10 [a]	2.03 ± 0.22 [a]	1.96 ± 0.14 [a]	1.78 ± 0.10 [a]
Dry matter (%)					
Diets	92.19 ± 1.16 [a]	94.46 ± 0.44 [a]	93.72 ± 0.29 [a]	93.68 ± 0.76 [a]	92.20 ± 1.14 [a]
Faeces	89.22 ± 1.44 [a]	90.30 ± 0.28 [a]	90.88 ± 0.39 [a]	91.46 ± 0.33 [a]	92.45 ± 0.19 [a]
Crude protein (%)					
Diets	41.72 ± 0.89 [a]	40.44 ± 0.87 [a]	42.16 ± 0.55 [a]	41.12 ± 0.30 [a]	41.34 ± 0.66 [a]
Faeces	1.92 ± 0.27 [a]	1.86 ± 0.10 [a]	2.03 ± 0.22 [a]	1.96 ± 0.14 [a]	1.78 ± 0.10 [a]
ADMD (%)	76.47 ± 3.46[a]	77.03 ± 1.22[a]	76.56 ± 2.63[a]	76.50 ± 1.76[a]	76.16 ± 1.31[a]
APD (%)	82.00 ± 2.65[a]	81.04 ± 1.00[a]	79.67 ± 2.28[a]	79.96 ± 1.50[a]	81.66 ± 1.01[a]

[1] Values are the mean for three replicates. Means in the same row with the same superscripts are not significantly different (P>0.05)

[2] Protein ratio of soybean meal (SBM) to azolla meal (AZM) (%)

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

Period	% Protein replacement of soybean meal by azolla meal				
	Diet RSA 0	Diet RSA25	Diet RSA50	Diet RSA75	Diet RSA100
30 min	4.40 ± 1.10[a]	4.15 ± 0.79[a]	4.24 ± 0.68[a]	4.59 ± 0.58[a]	4.45 ± 0.15[a]
60 min	6.98 ± 1.37[a]	7.94 ± 1.08[a]	7.78 ± 0.66[a]	8.18 ± 0.28[a]	7.81 ± 1.17[a]
120 min	7.08 ± 1.20[a]	7.65 ± 0.50[a]	8.42 ± 0.94[a]	8.28 ± 1.42[a]	9.82 ± 0.78[a]
180 min	8.48 ± 0.32[a]	11.20 ± 1.34[b]	9.15 ± 1.34[ab]	8.76 ± 1.16[a]	13.93 ± 1.18[c]
240 min	10.67 ± 1.09[a]	11.98 ± 0.85[a]	12.31 ± 0.68[ab]	10.71 ± 0.79[a]	13.93 ± 1.18[b]

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

In similar studies, Satoh *et al*. (1992) also proved that different faecal collection times from 3 to 15 hours after the final feeding had no significant effects on the apparent feed digestibility coefficients. In the present study, the maximum period faecal materials that was left in seawater was 4 hours. This period was within the limits outlined by other workers, suggesting that nutrient leaching was unlikely to have caused significant errors in digestibility determinations.

Digestibility coefficients of dry matter and protein for all experimental diets used in this study are similar to those reported in the literature for *P. monodon* diets based on soybean meal or fish meal (Catacutan, 1991; Eusebio, 1991; Shiu *et al.*, 1991; Shiau and Peng, 1992; Sudaryono *et al.*, 1996; Sudaryono, 1998). The data indicate that juvenile *P. monodon* can efficiently digest diets containing azolla meal regardless of its inclusion levels. The data also show that in terms of diet digestibility performance, the nutritive value of azolla meal is comparable to that of soybean meal for juvenile *P. monodon*. It means that azolla meal can compete successfully with soybean meal in practical diets for juvenile *P. monodon*.

The APD coefficients of all the practical diets obtained in the study were high with a range 79.7–82.0%. These values are also similar to those previously obtained by other workers for *P. monodon* fed practical diets based on various marine animal and plant protein (Catacutan, 1991; Eusebio, 1991; Shiau *et al*., 1991; Shiau and Peng, 1992; Sudaryono *et al.*, 1996,; Sudaryono, 1998). The data obtained in the present study indicate that protein of azolla meal is highly digestible for juvenile *P. monodon*. High protein digestibility of the diets in the present study suggests that any antinutritional factors such as trypsin inhibitor present in the diets, had little effect on digestive enzymes. The results are also exiting, azolla meal

has a high potential as alternative plant protein source to soybean meal in diets for *P. monodon*. This, in fact, may reduce a dependence of using soybean meal in aquaculture feeds due to it has been recognized by many workers in aquaculture nutrition as the most widely used plant protein source, beacuase of its favourable protein level and availability.

It is generally known that the physical quality of a pellet, especially its water stability, is affected by compositition of the feed and the processing method employed. Aadition 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 (Table 1), 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 similar to all test diets. Water stability of all diets after exposure time in seawater more than 120 min tends lower. The reduced water stability over the 480–minutes immersion period in seawater for all diets was likely to be due to the reduced binding characteristics of the ingredients in relation to an increase in exposure time period in seawater (Table 3). This reduced binding characteristic may have attributed to relatively high lipid contents of the diets (9.54%). This is in agreement with Lim and Cuzon (1994) explaining that a high dietary lipid content can reduce pellet water stability. A shrimp feed should contain lipid no more than 8% (Akiyama and Dominy, 1991).

Although there was no a significant difference in various replacement levels of dietary soybean meal protein with azolla meal, a bit trend of increased dry matter weight loss percentage of the test diets occurred when the dietary azolla meal contents gradually increase. This may be attributed to the reduced dietary starch contents as a binding agent. A low starch content of diets can be a reduced gelatinizing characteristic (Cuzon *et al*., 1994) as shown in Table 3 especially for diet RSA 100. High dietary fiber contents may also be a factor to lower pellet water stability. Fox et al. (1994) found that the diets with higher fiber contents (7.1–8.4%) had lower pellet water stability than those with lower fiber contents (5.1–6.4%). Overall, all test diets had good pellet water stability as the practical diets for juvenile shrimp *P. monodon* where, in fact, the diets exposed in seawater for over 8 hours have maximum dry matter weight loss of 15%.

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