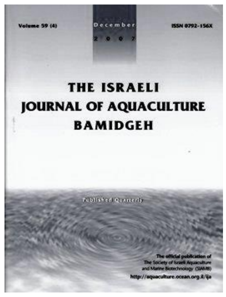
The *IJA* appears exclusively as a peer-reviewed on-line open-access journal at http://www.siamb.org.il. To read papers free of charge, please register online at registration form.
Sale of *IJA* papers is strictly forbidden.



Reduction of Particle Size of *Rhizoclonium riparium* Protein Concentrate Improves Digestibility, Growth, and Feed Efficiency of *Penaeus vannamei* Post Larvae

Mary Grace C. Sedanza[1], Barry Leonard M. Tumbokon[2], Augusto E. Serrano, Jr.[1,2]*

[1] *Institute of Aquaculture, College of Fisheries and Ocean Sciences, University of the Philippines Visayas, Miagao, Iloilo, Philippines*

[2] *National Institute of Molecular Biology and Biotechnology, University of the Philippines Visayas, Miagao, Iloilo, Philippines*

Keywords: acid-insoluble ash; milling; crinkle grass; apparent digestibility coefficient for dry matter; white shrimp

Abstract

The present study evaluated the effect of particle size (PS) of *Rhizoclonium* protein concentrate (RPC) on apparent digestibility coefficients (ADC) as well as on growth and feed efficiency of Pacific white shrimp (*Penaeus vannamei)* for a period of 30 days. Results showed that white shrimp could effectively digest *Rhizoclonium* protein concentrate in their diet. Average ADC values for dry matter (ADMD), crude protein (ADCP), and crude lipid (ADCL) were 71.1%, 84.4%, and 94.6%, respectively. A significant inverse relationship (p<0.05) was estimated between PS and ADMD (r=-0.66, p= 0.02). Particle size of 150 µm resulted in the highest final body weight (FBW), weight gain (WG), specific growth rate (SGR), feed conversion efficiency (FCE) and protein efficiency ratio (PER). All response parameters exhibited significant inverse correlations with particle size (p<0.05). In conclusion, digestibility of seaweed protein concentrate and its nutritive value could be improved by grinding it to an optimum size of 150 µm before dietary inclusion.

* Augusto E. Serrano, Jr., e-mail: aeserrano@up.edu.ph

Introduction

Rhizoclonium sp. is a cosmopolitan filamentous green alga that grows abundantly in brackishwater fishponds and poses a great threat to cultured species, as it removes dissolved oxygen, reduces space for cultured animals, and hinders their growth (Caffrey, 1992). The possible utilization of this nuisance seaweed as a feed ingredient whether as a meal or protein concentrate may create a demand for it and at the same time it could potentially replace imported ingredients thus lowering the cost of feeds.

An important factor in evaluating ingredients for use in aquaculture feeds is digestibility (Glencross et al, 2007; Serrano et al, 2015; Sedanza et al., 2016). Apparent dry matter digestibility (ADMD) provides a measure of the total quantity of an ingredient that is digested and absorbed. ADMD coefficients give a better estimate of the quantity of indigestible material present in a feed ingredient, since not all components of a feedstuff are digested equally as compared to the digestibility coefficients for individual nutrients, which in some cases are minimally affected by indigestible material in the diet (Santizo et al, 2014; Brunson et al 1997; Cho & Kaushik, 1990).

It is a commonly accepted hypothesis that smaller particles have increased surface area allowing the digestive enzymes to access the feed substrate, act on it, and effectively release nutrients. This might result in higher digestibility coefficients and improved feed efficiency in shrimp such as *Penaeus vannamei*. Studies which relate particle size reduction to digestibility of nutrients and growth performance are limited to terrestrial farm animals. No study has been reported on the effect of particle size reduction on the digestibility of feed ingredients in *P. vannamei.* Hence, such effects of the particle size (PS) of RPC were measured in *Penaeus vannamei* in this study.

Materials and Methods

The apparent digestibility coefficients of dry matter (ADMD), crude protein (ADCP), and crude lipid (ADCL) for RPC meal in Pacific white shrimp was determined using a test diet with 4 graded levels of particle sizes (PS) (Table 1). Diatomaceous earth, which is a form of acid insoluble ash, was used as an inert marker at a concentration of 10g/kg. There were four treatments with four replicates each.

Diet preparation. Composition of the formulated diet for both the digestibility and growth trial of Pacific white shrimp (Table 1) was according to those of Bunda et al. (2015) and Santizo et al. (2014). Diets were formulated with supplemental RPC meal ground to 4 different PS of identical proportions. For the growth trial, the formulation was as shown in Table 1. The ingredients were purchased from the Southeast Asian Fisheries Development Center-Aquaculture Department (SEAFDEC AQD) and were prepared at the Fish Nutrition Laboratory of the University. Prior to mixing, all ingredients except the RPC, were sieved through a 150μm sieve. Test diets containing graded PS of RPC were: Diet A (150 μm), Diet B (420 μm), Diet C (710μm) and Diet D (1400 μm). All dry ingredients were first thoroughly mixed before adding the liquid components (i.e. oil and lecithin). Then, the cooked bread flour (1:4 flour to water, respectively) was added. The resulting dough was pelleted using a meat grinder with a 2mm die, and oven dried for 24h at 60°C. The resulting hard noodle-like extrusions were stored to -20 °C prior to cutting into appropriate sizes (~4mm).

Table 1. Composition of the test diets containing graded PS of RPC for the *in vivo* digestibility and growth experiments in the Pacific white shrimp *Penaeus vannamei* (g/kg)

Ingredients	*Test diet (g/kg)*			
Danish Fish Meal	132.00			
Acetes	72.00			
Squid Meal	95.00			
Soybean Meal	200.00			
Bread Flour	35.00			
Rice Bran	100.50			
Wheat Pollard	100.00			
Cod Liver Oil	115.00			
Lecithin	10.00			
Ligno bond	10.00			
Vitamin Mix[a]	10.00			
Mineral Mix[b]	10.00			
BHT	0.50			
Diatomaceous earth[c]	10.00			
Rhizoclonium Meal (RM)	100.00			
TOTAL	1000.00			
Proximate Composition (%DM):	*Diet A (150 μ)*	*Diet B (420 μ)*	*Diet C (710 μ)*	*Diet D (1,400μ)*
Moisture	4.23	3.39	4.42	2.86
Crude protein	37.2	38.0	37.6	37.1
Crude lipid	14.6	14.7	13.9	12.2
Crude fiber	1.69	1.96	2.12	2.57
Ash	15.9	14.9	15.2	15.6
Nitrogen free extract	30.6	30.5	21.2	32.6
Total	100.0	100.0	100.0	100.0

[a]Vitamin mix: Vitamin A, 1 200 000 IU/kg; Vitamin D_3, 200 000 IU/kg; Vitamin E, 20 000 IU/kg; Vitamin B_1, 8 000 mg/kg; Vitamin B_2, 8000 mg/kg; Vitamin B_6, 5000 mg/kg; Vitamin B_{12} 1%, 2000 mcg/kg; Niacin, 40000 mg/kg; Calcium Pantothenate, 20000 mg/kg; Biotin, 40 mg/kg; Folic Acid, 1800 mg/kg; Ethoxyquin, 500 mg/kg
[b]Mineral mix: Fe, 40000 mg/kg; Mn, 10000 mg/kg; Zn, 40000 mg/kg; Cu, 4000 mg/kg; Cu, 4000 mg/kg; I, 1800 mg/kg, Co, 20 mg/kg; Se, 200 mg/kg
[c]Diatomaceous earth: 1.0%/kg; Nature's Wisdom; food grade

Rearing and feeding. A total of 64 mixed sex juvenile shrimps *Penaeus vannamei* (average body weight = 12.50 ± 1.41 g) were randomly stocked in a static system with individual aeration in sixteen 50L plastic aquaria. Sixteen juvenile shrimps were assigned to 4 replicate tanks. Salinity was maintained at 20-25ppt; all other water parameters were monitored regularly and continuously remained within ranges optimum for the white shrimp. The shrimp were acclimatized in the tanks by feeding the basal diet at 4% of body weight 3 times daily (0800, 1200, and 1600) for 3 days prior to receiving the experimental diets, and 4 more days prior to collection of fecal matter.

Fecal collection. The growth trial and collection of feces were conducted simultaneously and lasted for 30 days in order to obtain sufficient fecal samples. Shrimp were fed the test diets 3 times a day at 0800, 1200 and 1600 at 4% body weight. Feeding lasted for 45 min and uneaten feed was siphoned off. Fecal collection began 4 days after feeding the shrimp with their respective test diets to allow evacuation of previously ingested material (Goddard and McLean, 2000). Intact fecal strands were carefully pipetted into a container fitted on top with a fine mesh plankton net and rinsed once with distilled water; the pipetting was done every 30 min for the next 3 h to reduce nutrient leaching. Fecal matter was pooled for each dietary treatment, dried at 60°C for 24 h, finely ground using a commercial coffee grinder (Krups F203), and stored at -20°C until chemical analysis.

Calculations of growth and feed efficiency. Shrimp were weighed at the start and end of the experiment to determine weight gain (WG), specific growth rate (SGR), feed conversion efficiency (FCE) and protein efficiency ratio (PER) according to Hardy and Barrows, (2002) and the apparent digestibility coefficient (ADC) for dry matter (ADMD), crude protein (ADCP) and crude lipid (ADCL) according to Goddard and McLean (2000):

$$WG(g) = FBW - IBW$$

$$SGR\ (\%/day) = 100*(\ln FBW - \ln IBW)/D$$

$$\%\ FCE = \frac{(FBW-IBW)}{FI} \times 100$$

$$PER = \frac{(FBW-IBW)}{(FI \times FP)}$$

$$\%ADC = 100*(1-(Fn \times Dn)/(Dm \times Fm))$$

Where:
FBW = final body weight (g) of individual fish
D = days of culture
IBW = initial body weight (g) of individual fish
FP= Feed Protein (in decimal)
CP_i= Initial carcass protein (in decimal)
CP_f= Final carcass protein (in decimal)
FI= total feed intake of individual fish for the whole duration of the experiment
Fn = % nutrient in feces
D = % nutrient in diet
Dm = % marker in diet
Fm = % marker in feces

Chemical analysis. Diets and fecal samples were analyzed at the Laboratory Facilities for Advanced Aquaculture Technologies (LFAAT) SEAFDEC, Iloilo for proximate analysis. Analysis of the acid insoluble ash (AIA) was conducted as described by Atkinson et al. (1984) and was performed at the University laboratory. Approximately 2 g of feed and fecal samples were placed in a muffle furnace for 2 h at 600°C. The resulting ash was boiled in 75 ml of 2M HCl for 5 min, filtered through ashless filter paper (Whatman no. 41), the residue washed with boiling distilled water, and allowed to dry. The dried filter paper with the residue was washed again for 2 h at 600°C and the resulting material was regarded as the AIA content of the feeds or feces.

Statistical analyses. Data on growth, feed efficiency, nutrient utilization, and digestibility were subjected to the Shapiro-Wilk test for normal distribution and to the Levene's test for homogeneity of variances. Data that passed these tests were subjected to one way Analysis of Variance (ANOVA); those that did not were transformed until they passed both tests and were subsequently subjected to one-way ANOVA test. Tukey's test post hoc analysis was performed at α=0.05 to determine the ranks of the means.

Results

Apparent Digestibility Coefficients for dry matter (ADMD), crude protein (ADCP), and crude lipid (ADCL) of the 4 diets used in the study are shown in Table 2. All diets with varying RPC PSs (150 µm, 420 µm, 710 µm, and 1,400 µm) were well digested by the white shrimp. ADMD and ADCP values of shrimp fed diets containing PS of 150 µm, and 420 µm RPC meal, were significantly higher than those fed diets containing larger PS (710 µm and 1400 µm) of RPC. In contrast, ADCL was not affected by the PS of the RPC meal.

Table 2. Apparent digestibility coefficients for dry matter (ADMD), crude protein (ADCP), and crude lipid (ADCL) of diets for the white shrimp *Penaeus vannamei* (Mean ± SEM*).*

	ADMD	*ADCP*	*ADCL*
Diet 1 (150 μm)	75.7 ± 1.2[c]	86.6 ± 0.67[b]	96.7 ± 0.21[a]
Diet 2 (420 μm)	73.6 ± 2.5[bc]	85.0 ± 1.3[ab]	92.7 ± 2.6[a]
Diet 3 (710 μm)	66.6 ± 0.44[a]	83.0 ± 0.23[a]	94.8 ± 0.0[a]
Diet 4 (1,400μm)	68.3 ± 0.82[ab]	82.8 ± 0.38[a]	94.3 ± 0.23[a]

ADMD = apparent dry matter digestibility; ADCP = apparent digestibility of crude protein; ADCL = apparent digestibility of crude lipid.

For the growth trial, the diet containing 150 μm of RPC meal (Diet 1) resulted in the highest final body weight (FBW), weight gain (WG), specific growth rate (SGR), feed conversion efficiency (FCE) and protein efficiency ratio (PER) than the other diets containing larger PS (Table 3). Growth performance and feed efficiency of *P. vannamei* exhibited significant inverse correlation (p<0.05) with the PS of the seaweed concentrate (Table 4). Survival rate was excellent (100%) in all treatments for the duration of the experiment.

Table 3. Growth Performance of Pacific white shrimp, *Penaeus vannamei* fed diets containing graded particle sizes of the *Rhizoclonium* protein concentrate for 30 days (% ± SEM).

Diets	*FBW*	*WG*	*SGR*	*FCE*	*PER*
Diet 1 (150 μ)	18.5 ± 0.0[c]	5.46 ± 0.02[c]	1.17 ± 0.0[c]	0.52 ± 0.0[c]	1.39 ± 0.01[c]
Diet 2 (420 μ)	17.2 ± 0.44[bc]	4.18 ± 0.21[b]	0.93 ± 0.04[b]	0.39 ± 0.02[b]	1.03 ± 0.05[b]
Diet 3 (710 μ)	16.4 ± 0.07[ab]	3.43 ± 0.20[ab]	0.79 ± 0.06[ab]	0.33 ± 0.02[ab]	0.87 ± 0.05[ab]
Diet 4 (1,400 μ)	15.7 ± 0.44[a]	2.79 ± 0.26[a]	0.65 ± 0.06[a]	0.26 ± 0.02[a]	0.71 ± 0.06[a]

Mean values with the different superscripts are significantly different (p<0.05). FBW = final body weight (g) ; WG = weight gain (g); SGR = specific growth rate (% body weight/day); FCE = food conversion efficiency; PER = protein efficiency ratio.

Table 4. Matrix of two-tailed Pearson correlation moment (r) of the particle size (PS) of the RPC meal with values of the apparent digestibility coefficient, growth and feed efficiency (N=12, α=0.05).

	ADMD	*ADCP*	*FBW*	*WG*	*SGR*	*FCE*	*PER*
PS	-0.66	-0.70	-0.85	-0.89	-0.89	-0.90	-0.88
p	*p*=0.02	*p*=0.01	*p*=0.00	*p*=0.000	*p*=0.00	*p*=0.00	*p*=0.00

PS = particle size; p = probability; ADMD = apparent dry matter digestibility; FBW = final body weight; WG = weight gain; SGR = specific growth rate; FCE = feed conversion efficiency; PER = protein efficiency ratio

Discussion

In the present study, the linear decrease in PS of RPC resulted in the linear increase in ADMD, ADCP and ADCL of the concentrate in the white shrimps. Probable reasons for this observation could be that smaller particles had greater total surface area allowing for greater penetration of the digestive enzymes into the feed ingredient, enabling them to act on the feed substrate efficiently and effectively release nutrients. These findings generally result in higher digestibility coefficients and improved feed efficiency responses in other animals (Owsley et al., 1981).

The highest FBW, WG, SGR, FCE and PER were observed in shrimps fed the diet with the lowest PS of 150μm of the RPC. Results of the present study demonstrated that a decrease in PS of a feed ingredient enhanced growth and feed efficiency performance of *Penaeus vannamei* without manipulating the composition of the formulated diet. The optimal PS found in the present study was close to that of the findings of Obaldo et al. (2002) in shrimps, i.e. milling feedstuffs to 124 μm particle size before pellet manufacture influences pellet water stability, pellet durability, starch gelatinization, shrimp live weight, and weekly weight gain. One study recommended that 95 % of the feed ingredients in crustaceans should be less than or equal to 250 μm and for smaller shrimp, 150 to 180 μm (Tan and Dominy 1997). Moreover, these authors observed that

PS bigger than 150 μm results in insignificant differences in growth performance of shrimp with no deleterious effect on survival.

In conclusion**,** the ADMD of RPC meal was improved from 68.3% to 75.7% while enhancing the growth and feed efficiency of the Pacific white shrimp Penaeus vannamei by merely decreasing the PS from 1,400 μm to 150 μm.

Acknowledgements

The authors wish to express their gratitude to the Philippine Department of Science and Technology (DOST) Accelerated Science and Technology Human Resource Development Program (ASTHRDP) for the scholarship provided to Ms. Mary Grace C. Sedanza and for the additional publication support. They are also grateful to the DOST-Philippine Council for Agriculture, Aquatic and Natural Resources Development (PCAARD) and the UPV Office of the Research and Extension for the partial research funding and publication support.

References

Atkinson J. L., Hilton J. W., Slinger S. J., 1984. Evaluation of acid-insoluble ash as an indicator of feed digestibility in rainbow trout (*Salmo gairdneri*). *Canadian J. Fish. Aquac. Sci.,* 41:1384 – 1386.

Brunson J. F., Romaire R.P., Reigh R.C., 2003. Apparent digestibility of selected ingredients in diets for white shrimp *Penaeus setiferus* L. *Aquac. Nutr.,* 3(1):9-16.

Bunda M. G. B., Tumbokon B. L. M., Serrano Jr. A. E., 2015. Physical properties of *Rhizoclonium* meal as feed ingredient in aquafeeds. *ABAH Bioflux*, 7(1):79-86.

Caffrey J. M., 1992. Management of aquatic and invasive riparian vegetation in Irish amenity watercourses. In: *Water of Life*. Mollan C. (ed.), Royal Dublin Society, Dublin, pp. 130-141.

Cho C. Y., Kaushik S. J., 1990. Nutritional energetics in fish: energy and protein utilisation in rainbow trout (*Salmo gairdnerii*). *World Rev. Nutr. Diet*., 61:132–172.

Glencross B. D., Booth M., Allan G. L., 2007. A feed is only as good as its ingredients – a review of ingredient evaluation strategies for aquaculture feeds. *Aquac. Nutr.* 13:17-34.

Goddard J. S., McLean E., 2001. Acid- insoluble ash as an inert reference material for digestibility studies in tilapia, O*reochromis aureus. Aquaculture*, 194: 93-98.

Hardy R. W., Barrows F. T., 2002. *Fish Nutrition*. 3rd edition. In: J.E. Halver and R. W. Hardy (eds.). Academic Press, San Diego, California, USA, pp. 588 – 591.

Obaldo L. G., Divakaran S., Tacon A. G., 2002. Method for determining the physical stability of shrimp feeds in water. *Aquac. Res.,* 33(5):369-377.

Owsley, W. F., Knabe, D. F., Tanksley, T. D., 1981. Effect of sorghum particle size on digestibility of nutrients at the terminal ileum and over the total digestive tract of growing-finishing pigs. *J. Anim. Sci.,* 52(3):557-566.

Santizo R., Serrano Jr. A. E., Corre V. L., 2014. Proximate composition and dry matter digestibility of *Ulva lactuca* in the black tiger shrimp *Penaeus monodon*. *ABAH Bioflux*, 6(1):75- 83.

Sedanza M.G., Serrano, Jr. A.E*. 2016. Evaluation of the Crinkle Grass Rhizoclonium riparium in the diet of the Pacific White Shrimp Penaeus vannamei post larvae, 7 pages. *Isr. J. Aquacult.-Bamidgeh,* IJA_68.2016.1328.

Serrano Jr. A. E., Declarador R., Tumbokon B. L. M., 2015. Proximate composition and apparent digestibility coefficient of *Sargassum spp.* meal in the Nile tilapia, *Oreochromis niloticus. ABAH Bioflux*, 7(2):159-166.

Tan R. K. H., Dominy W. G., 1997. Commercial pelleting of crustacean feeds. In: L.R. D'Abramo, D.E. Conklin and D.M. Akiyama (eds.) *Crustacean nutrition*, 6:520–549. Baton Rouge, World Aquaculture Society.