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Growth performance of *Penaeus monodon* fed diets containing water hyacinth leaf protein concentrate

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Abstract. Growth performance and nutrient utilization of *Penaeus monodon* post larvae (5 ± 0.0031 mg) fed diets containing increasing levels of water hyacinth leaf protein concentrate (WHLPC) (0 %, 25 %, 50, and 75 %) replacing soybean meal dietary protein were assessed. Each diet was fed to triplicate groups of shrimp for sixty days. Survival, percent weight gain, specific growth rate, and protein efficiency ratio were measured. Highest survival (93 %), percent weight gain (2,423), specific growth rate (4.29) and protein efficiency ratio (0.55) was recorded in shrimp fed diet containing 50 % WHLPC dietary protein. However, all values obtained showed no significant difference among all treatments. The present results indicate that soybean meal dietary protein can be replaced by WHLPC until 75 % without affecting the overall performance of *P. monodon* post larvae.

Key Words: Dietary protein, giant tiger prawn, Asian tiger shrimp, protein efficiency ratio, growth rate, dietary protein replacement, survival rate.

Introduction. Currently, aquaculture industry is dependent on imported feed ingredients including soybean meal, fishmeal, and fish oil for feed production. Further, the costs of these key feed components are rising due to the increasing global demands caused by usage competition among the animal growing industries and is hypothesized to hamper the growth and expansion of the industry. The use of locally produced plant-derived feed ingredient as full or partial substitute for soybean and fishmeal on aquaculture feed could be a potential approach to lessen dependence on imported and costly feed inputs (Gatlin et al 2007). Concentrated protein derived from aquatic plants has potential as aquaculture feed ingredient. Earlier scientific reports indicate that protein concentrate sourced from plant materials can be well utilized by animals including fish. Protein concentrates derived from plant leaves have been tested as feed material for swine, cow, chicken, and fish (Telek & Graham 1983). Moreover, protein concentrates from rice bran, wheat, and corn have been tested and proven a feasible protein source that could satisfy the protein requirements of carp, turbot, and salmon (Kaur & Saxena 2005; Rónyai & Gál 2005; Robaina et al 1999).

Water hyacinth, an exotic species in the Philippines, has invaded natural bodies of water hampering the natural water-flow resulting to flashfloods during monsoon seasons. Recently, attention is being devoted to the utilization of water hyacinth since the efforts to control plant growth by chemical, biological and mechanical means have met with little success (Wolverton & McDonald 1979; IFPRI 2009). Because of its relatively high protein content and abundance in tropical and subtropical countries, a significant number of research studies have been carried out to find the potential for the utilization of water hyacinth as fish feed. Available literature indicates that water hyacinth is fed to fish and livestock either in fresh form, or as a dried meal in pelleted diets, or composted as feed (Polprasert et al 1994). Apart from these three forms, attempts were also made to feed water hyacinth to fish by processing them with other techniques. Many of these studies were conducted under laboratory conditions and reports on utilization as aquaculture feed are rather limited.

The present study aimed to evaluate water hyacinth leaf protein concentrate (WHLPC) as substitute to soybean meal protein in the diet of *Penaeus monodon* post larvae.

Material and Method

Experimental animals. Pathogen-free *P. monodon* post larvae (PL12, 5.0 mg) were obtained from a commercial hatchery in the province of Iloilo and transported to Institute of Aquaculture Multi-Species Hatchery (University of the Philippines Visayas, Miagao, Iloilo). Post larvae individuals were stocked and acclimated in 2-ton fiberglass tank indoors for 7 days. The subjects were fed artemia and commercial post larvae feed three times daily.

Experimental set-up. Twelve tanks were used to hold the test animals constituting the 4 experimental treatments in triplicate. Tanks were supplied with constant aeration maintaining oxygen near saturation levels. Water temperature was at the range of 28 °C to 30 °C, at a salinity of 32 to 35 psu and the animals were exposed to normal photoperiod.

Water temperature, salinity, dissolved oxygen (DO), and pH were monitored daily. Total ammonia nitrogen (TAN), ammonia, and nitrite-nitrogen were monitored and measured weekly using test kits (Advance Pharma Co., Ltd., Bangkok, Thailand).

Test diets. Four experimental diets (Table 1) were formulated to evaluate the effects of increasing protein substitution by water hyacinth leaf protein concentrate (WHLPC) to soybean meal at 0 %, 25 %, 50 %, and 75 %. The control diet (0 %) contained soybean meal, fishmeal and squid meal as the main protein sources.

All four diets were formulated to be isonitrogenous (40 % crude protein) and isolipidic (8 % crude lipid). Pelleted diets were prepared by mixing the dry ingredients with fish oil/soybean oil and added with cooked starch forming a dough. The dough was pelleted by passing through a meat grinder twice and the resulting strands were put in an oven at 60 °C until dry. After drying, strands were broken, sieved to a convenient size, and stored at 4 °C until use. At the beginning of the experiment, 250 – 350 μm crumbles were used, increasing its size up to 800 – 1000 μm at the end of the experiment.

Water hyacinth leaf protein concentrate (WHLPC) was produced following the method of Virabalin et al (1993). WHLPC used in this study was a by-product from water hyacinth leaves.

Table 1

Composition of experimental diets (g/100 g dry weight) used in feeding trial

Ingredient	*% Dietary protein replacement of soybean meal with WHLPC*			
	0 %	*25 %*	*50 %*	*75 %*
Soybean meal	30	22	14	8
WHLPC	0	18	35	48
Fish meal	20	20	20	20
Squid meal	14	14	14	14
Fish oil/soybean oil	5	4	3.5	3
Starch	20	14	8.98	2.48
Vitamin premix	2	2	2	2
Mineral premix	0.5	0.5	0.5	0.5
Lecithin	0.5	0.5	0.5	0.5
BHT	0.02	0.02	0.02	0.02
Lignobond	1.5	1.5	1.5	1.5
Alpha-cellulose	6.48	3.48	0	0
Total	100	100	100	100
Proximate analysis of shrimp diets (%)				
	0 %	25 %	50 %	75 %
Crude Protein	40.86	40.85	41.75	41.12
Crude Fat	9.11	8.74	8.89	8.83
Ash	10.20	10.66	11.13	12.27
Dry Matter	94.41	94.14	93.44	92.6

Feeding trial. Following the acclimation, the two hundred forty postlarvae were randomly collected and distributed in 12 tanks (30 L volume). Animals were fed three times daily (9 AM, 1 PM, and 5 PM) following a fixed feeding regimen that was adjusted every ten days during sampling. Experimental diets were given to the treatment groups for 60 days culture period. Uneaten feed, feces, molts, and dead shrimp in each tank were collected and 30 % of the water was replenished daily prior to feeding. To assess growth rate, shrimp were bulk weighed per tank every ten days throughout the trial. At the end of the feeding trial, shrimp were harvested, enumerated, blotted dry and weighed individually by treatment tank.

Biological parameters used to evaluate the quality of experimental diets were calculated by equations as follows:

- Percent Weight Gain (% wt gain) = [(final body weight – initial body weight) / initial body weight] x 100
- Specific Growth Rate (SGR) = [(ln average final weight – ln average initial weight)/number of days] x 100
- Feed Conversion Efficiency (FCE) = 1 / [weight gain (g) / dry feed intake (g)]
- Protein Efficiency Ratio (PER) = weight gain (g) / protein intake (g)
- Survival (%) = (Final number of shrimp/ Initial number of shrimp) x 100
- Protein Retention = [Protein gain in fish (g) / Protein intake in food (g)] x 100

- Lipid Retention = [Lipid gain in fish (g) / Lipid intake in food (g)] x 100

Statistical analysis. All data were statistically analyzed by the Statistical Analysis Program of SPSS Inc. One Way ANOVA and the Tukey's Test at $P<0.05$ confidence level.

Results. Percent weight gain of the test shrimp increased at every sampling period. At the end of the feeding trial, weight gain was found to be higher in treatments receiving the diets with WHLPC as a replacement for soybean meal however; statistical analysis indicates that this value is not significantly different with that of the control diet (0%) with no WHLPC. Survival rates was higher in shrimp fed with WHLPC with the highest survival rate (93.33 %) recorded at 50 % dietary protein replacement (Table 2).

Shrimp growth performance in terms of SGR and PER showed no significant decline as WHLPC replacement increases. Biological growth indices were similar in dietary treatments with 75 % replacement level and those that received the full soybean meal diet as the control group. Highest PER value (0.55 ± 0.04) was observed at 50 % WHLPC dietary protein replacement compared to that of shrimps receiving the control diet , 0.45 ± 0.04 (Figure 2). However, no significant difference was found among all treatments in terms of PER.

Group fed the diet containing 25 % WHLPC dietary replacement recorded the lowest FCR at 4.12 ±0.46 but showed no significant difference ($p>0.05$) with all other diets. Group fed highest WHLPC replacement exhibited the lowest feed efficiency. Levels of WHLPC dietary protein replacement did not have significant influence on protein retention of *P. monodon* postlarvae. However, lipid retention in shrimp fed diet with increasing replacement levels were found different ($p<0.05$) among treatments. Highest lipid retention (0.74 ± 0.09) was in shrimp fed diet with 50 % WHLPC replacement but was not significantly different from shrimp fed diet containing 75 % WHLPC.

Discussions. Water hyacinth leaf protein concentrate has the potential to be used as feed protein source for shrimp and to our knowledge, this ingredient has not been evaluated in previous works. In earlier studies, water hyacinth was used as feed in the form of leaf meal or silage in livestock. Like other feedstuffs, acceptability and suitability of WHLPC would vary by species. Liang & Lovell (1971) were the first to test the use of water hyacinth as feed ingredient in rations of channel catfish. Hyacinth protein extract was produced and incorporated in increasing amounts in the diets with 40 % as the highest inclusion level. Optimum replacement level of water hyacinth protein extract that promoted biological growth response similar to the control diet was at 5 - 10 % of the diet.

Present results agree favorably with Agbo et al (2011), who reported that growth performance of tilapia fed with groundnut by-product-based diets has higher growth values than that of the control but was not significantly different. However, 50 % inclusion of groundnut cake resulted in deficiency of three EAAs (lysine, methionine, and threonine). Nyina-wamwiza et al (2007) obtained same results wherein 50 % is the optimum replacement of fishmeal with groundnut cake in *Clarias* fingerling diets.

Table 2

Biological responses of *Penaeus monodon* fed diets with WHLPC as soybean meal replacement

WHLPC dietary protein replacement	*% Weight gain*[2]	*Survival rate*[3]	*SGR*[4]	*PER*[5]	*FCE*[6]	*Protein retention*[7]	*Lipid retention*[8]
0 %	1881.76 ± 280.56	77.78 ± 1.11^{b}	3.95 ± 0.11	0.45 ± 0.04	0.23	7.06 ± 1.02	0.38 ± 0.06^{b}
25 %	1779.67 ± 67.55	85.56 ± 2.94^{ab}	3.91 ± 0.03	0.45 ± 0.05	0.21	6.03 ± 0.22	0.35 ±0.01^{b}
50 %	2423.53 ± 290.73	93.33 ± 3.85^{a}	4.29 ± 0.09	0.55 ± 0.04	0.24	9.04 ± 1.05	0.74 ± 0.09^{a}
75 %	1725.00 ± 158.33	86.67± 3.33^{ab}	3.86 ± 0.06	0.39 ± 0.03	0.18	6.61 ± 0.58	0.58 ± 0.05^{ab}

[1]Values are means of three groups of shrimp (n=3), with 20 shrimp per group. Values within similar rows with different letter superscripts are significantly different.

Results of the present study indicate that substitution of soybean meal dietary protein up to 75 % with WHLPC did not affect shrimp performance. These results conform to the reports of others that plant protein can be successfully included in practical aquaculture diets. When alternate plant protein sources are used in diets containing the same concentration of digestible energy and protein and are able to meet the nutritional requirements of the animal being fed, similar performance may be expected (Cruz-Suarez et al 2001).

Generally, reduced growth performance of test animals fed with plant-based protein diets at higher inclusion rates may be attributed to essential amino acids deficiency or the presence of anti-nutritional factors, low palatability, and digestibility. In this study, the utilization of more than 10 % of marine protein ingredients (squid meal and fishmeal) most likely facilitated higher soybean meal replacements. Moreover, questions remain regarding the palatability and amino acid availability of plant-based products (Li et al 2004). Challenges also are met when considering vegetable protein sources, especially for use in feeds for higher-level carnivores. Most plant proteins harbor anti-nutritional factors and have low biological value due to essential amino acid deficiencies and/or imbalances and poor digestibility (Hardy 1996; Francis et al 2001). In the present study, no significant decline in growth performance was observed even at the highest level of replacement. Collectively, this indicates that WHLPC does not contain anti-nutritional factors that may affect growth.

Conclusions. This study has demonstrated the optimum inclusion of WHLPC as feed ingredient in shrimp diets, since this product can replace the commonly used shrimp feed ingredient, soybean meal. Water hyacinth leaf protein concentrate can replace 75 % dietary protein of soybean meal in formulated diets for *P. monodon* without adversely effecting growth and survival.

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