AACL BIOFLUX

Aquaculture, Aquarium, Conservation & Legislation
International Journal of the Bioflux Society

Potential use of the sea lettuce *Ulva lactuca* replacing soybean meal in the diet of the black tiger shrimp *Penaeus monodon* juvenile

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Abstract. To evaluate the biological value of incorporating the sea lettuce *Ulva lactuca* meal in the diet of the black tiger shrimp (*Penaeus monodon*), 3 diets were fed to groups of shrimps containing two levels (15% and 30% replacement of soybean meal) of the sea lettuce for 90 days. Biological parameters were determined either periodically or at the termination of the experiment. Specific growth rate (SGR) of shrimp fed the control diet and those fed with the diet containing 15% replacement were not significantly different from each other while that of shrimp fed 30% soybean replacement was slightly but significantly inferior. All other parameters such as survival rate, feed intake, food conversion efficiency, protein efficiency ratio protein and lipid deposited and body composition were all statistically similar between the experimental groups of shrimp. Thus, the 30% replacement level or 10.5% inclusion level could be used in the diet of the shrimp *P. monodon*. When performances were compared with the best result in incorporating *U. lactuca* protein concentrate from a previous study and that in the present study (both were 30% replacement or 10.5 inclusion level), they were statistically similar. Thus, the raw *U. lactuca* meal is recommended because it did not require additional processing to produce the concentrated seaweed.
Key Words: seaweed, replacement, soybean meal, feed performance, crustacean.

Introduction. In the diet of terrestrial animals, macroalgae has been utilized to replace animal ingredients for years (Leupp et al 2005). *Ulva lactuca* and *Ulva rigida*, among a few seaweeds, are potential alternative source of nutrients for aquafeeds (Valente et al 2006). Even if they contain low protein (about 5–30% of the dry weight) (Anh et al 2013) their advantages include high nutritional value, locally available in the Philippines as compared to the imported soybean in most Southeast Asian countries, cheap ingredient (Mustafa & Nakagawa 1995). They also contain all essential amino acids in various concentrations (Galland-Irmouli et al 1999), essential fatty acids (MacArtain et al 2007) and polysaccharides that have bioactive compounds (Burtin 2003; Declarador et al 2014; Serrano Jr. & Declarador 2014; Lauzon & Serrano Jr. 2015). In the shrimp *Litopenaeus vannamei*, seaweed supplementation in the diet has improved growth, feed utilization, carcass quality and immune response (Cruz-Suárez et al 2009). Another closely related species belonging to Ulvaceae is *Ulva clathrata* has been documented to contain carotenoids which contribute to the shrimp pigmentation, 30% lower hydrocolloid levels than *Macrocystis* and *Ascophyllum* (Cruz-Suarez et al 2009).

Previously, it was demonstrated that protein concentrate of the sea lettuce *U. lactuca* could be produced by chemical treatment (Santizo et al 2014). This process increased the protein content from 13.4% to 38.4% and decreased ash content from 31.7% to 14.7%. Its dry matter digestibility was increased from 71.5% to 98.8%. We also evaluated the growth performance of the shrimp *Penaeus monodon* fed diets containing the seaweed protein concentrate (Serrano Jr. & Santizo 2014). Survival was not affected but feed intake at higher levels (30% and 45% soybean replacement by weight) decreased significantly. Specific growth rates were similar in shrimps fed the control diet (no seaweed) and those fed diets containing the concentrated seaweed up to 30% soybean meal replacement. These beneficial effects of the *U. lactuca* were similar with other works on microalgal meal (not concentrate) (Mustafa & Nakagawa 1995; Mustafa et al 1995). This was contrary to the observation that the inclusion of *Gracilaria lemaneiformis* meal did not improve the growth the black sea bream, *Acanthopagrus schlegelii* juvenile (Xuan et al 2013). *U. lactuca* meal in the diet of *Oreochromis niloticus* (Guroy et al 2007) and *Ulva rigida* meal in the diet of the European sea bass *Dicentrarchus labrax* juveniles resulted in decreased growth performance and feed utilization. This study aims to evaluate the potential of the raw *U. lactuca* meal (ULM) as partial replacements to soybean meal in *Penaeus monodon* diets for growth and nutrient utilization efficiency.

Material and Method

Experimental animal and set up. The feeding trial was conducted at the Institute of Aquaculture Multispecies Hatchery in a recirculating system between September and December 2013. *P. monodon* juveniles (600 individuals) were obtained from the hatchery of the University of the Philippines Visayas in Miagao, Iloilo, Philippines. Prior to the conduct of the study, samples of shrimp were subjected to one-step Polymerase Chain Reaction (PCR) for the detection of White Spot Syndrome Virus (WSSV) and were found to be free from the pathogen. Shrimps were acclimated to the laboratory conditions for 2 weeks in a one-ton fiberglass-holding tank equipped with aeration at salinity range of 26-28 ppt. The shrimp were fed the control diet containing no seaweed (Table 1) 4 times a day. Water was replaced daily at a rate of 10-30%. Representative shrimp samples were collected, dried and stored in a freezer (-20°C) for initial proximate analysis of carcass. Shrimp juveniles (average body weight of 0.11 ± 0.02 g) were distributed randomly in 18 substrate-free 50-L culture tanks at a stocking density of 15 shrimp each tank. The shrimp were conditioned to the experimental condition for 5 days and fed with the control diet. There were 3 dietary treatments representing 3 levels (0, 15% and 30% soybean meal replacement by weight, Table 1) of the raw ULM. Two replacement levels by the seaweed meal represent a final dietary inclusion of 5.2% and 10.5% of the diet. A diet without the seaweed served as the control treatment. Dietary treatments were randomly assigned to 18 tanks with 3 replicates per dietary treatment. The feeding experiment was conducted for 90 days.

Feeding and management. Shrimps were fed at a sliding rate of 15%-3% of the average body weight divided into 4 equal feeding per day at 08:00, 11:00, 14:00 and 17:00. The fecal matter and uneaten feed were siphoned before feeding in the morning. Water temperature, salinity, dissolved oxygen, pH, nitrite and total amino nitrogen (TAN) were maintained at 26.5-31.0°C, 25-28 parts per thousand (ppt), 7-10 mg L^{-1}, 8.0-8.5, 0.05-0.10 parts per million (ppm), and 0.10-0.20 ppm respectively. Shrimps were weighed in bulk every 15 days and the amount of feed to be given after the sampling was estimated. Water from the chamber as well as the fiber filter was changed twice every week. To prevent the growth of algae, the experimental tanks were cleaned every day. At the end of the culture period, shrimp in every treatment were pooled, sacrificed and subjected to carcass proximate analysis.

Table 1
Composition of experimental diets containing raw *U. lactuca* meal (ULM) as a substitute for soybean meal in the diet of shrimp *P. monodon*

Ingredients	*D1 (0%)*	*D2 (15% ULM)*	*D3 (30% ULM)*
Danish fish meal	380.0	380.0	380.0
Squid meal	29.0	29.0	29.0
Soybean meal	350.0	298.0	245.0
Bread flour	80.0	80.0	80.0
Cod liver oil	63.0	63.0	63.0
Lecithin	5.0	5.0	5.0
[a]CMC	37.5	37.5	37.5
Ligno bond	15.0	15.0	15.0
[b]Vitamin mix	10.0	10.0	10.0
[c]Mineral mix	10.0	10.0	10.0
Dicalcium phosphate	20.0	20.0	20.0
[d]BHT	0.5	0.5	0.5
ULM	0.0	52.0	105.0
Total	1000.0	1000.0	1000.0
	Proximate analysis (%)		
Moisture	4.2	4.9	4.9
Crude protein	41.3	38.5	37.3
Crude fat	10.8	10.4	10.2
Crude fiber	2.5	2.2	2.7
[e]NFE	26.0	29.9	28.9
Ash	15.3	14.0	16.1
[f]Gross energy (KJ g^{-1})	18.4	18.3	17.7

[a] Carboxymethyl cellulose; [b]Vitamin mix (mg or IU kg^{-1} diet): Vitamin A - 12,000 IU; Vitamin D3 - 2,000 IU; Vitamin E - 200 IU; Vitamin B1 - 80; Vitamin B2 - 80; Vitamin B6 - 501; Vitamin B12 - 2000 mcg kg^{-1}; Niacin - 400; Calcium Pantothenate - 200; Biotin - 0.4; Folic Acid - 18 mg kg^{-1}; Ethoxyquin - 5; [c] Mineral mix (mg kg^{-1} diet): Fe - 400; Mn - 100; Zn - 400; Cu - 40; I - 18; Co - 0.2; Se - 2; [d] Butylhydroxytoluene; [e] Nitrogen free extract; [f] Gross energy estimated according to 23.6 KJ g^{-1} protein, 39.5 KJ g^{-1} lipid, and 17.0 KJ g^{-1} NFE (Ergun et al 2009).

Growth performance and feed utilization. Growth performance and feed utilization were evaluated using the following formula (Hardy & Barrows 2002):

Specific growth rate (SGR) (% day^{-1}) = (ln FBW – ln IBW)/D * 100

Where:
FBW = final body weight;
IBW = initial body weight;
D = number of days of rearing.

FCE = wet weight gain (g)/feed consumed (g);
PG (g) = (final-initial) whole body protein;
PER = wet body weight gain/protein intake;
Protein Retention = [{(% final carcass protein x final ABW (g)) – (% initial c carcass protein x initial ABW (g))} /total protein intake (g)] x 100;
Lipid Retention (%) = [{(% final carcass lipid x final ABW (g)) – (% initial carcass lipid x initial ABW (g))}/total lipid intake (g)] x 100;
Survival Rate (%) = 100 x (final number of shrimp/initial number of shrimp).

Chemical analysis. Experimental diets as well as the initial and final carcasses were analysed for proximate composition (AOAC 2002). Crude protein, crude lipid, crude fiber, NFE, moisture and ash were determined using Fibertech™ 1023 System E.

Statistical analysis. Statistical analysis was performed using Statistical Analysis Software Program (SPSS) version 16. Data were presented as mean ± standard error of the mean (SEM) for each treatment. Data were analysed for normal distribution using Kolmogorov-Smirnov test and Levene's test for homogeneity of variances. Data that did not pass these tests were transformed until they passed these tests. Data on digestibility, growth parameters, feed efficiency and nutrient utilization were subjected to one-way analysis of variance (ANOVA). When ANOVA result showed significant difference, Tukey's test was performed to determine the differences between the treatment means. Student t-test was employed in the paired comparison between the performance of the *U. lactuca* protein concentrate from the previous study (Serrano Jr. & Santizo 2014) and that of the present study. Difference was regarded as significant when $p < 0.05$.

Results and Discussion. Shrimp fed with the control diet and with Diet 2 (15% ULM) resulted in significantly higher SGR than those fed with 30% UM (Table 2). In terms of feed intake (FI), feed conversion efficiency (FCE), protein gained (PG), and protein efficiency ratio (PER), no significant differences were observed in all treatments. Survival rate of shrimp ranged from 87% to 93% at the end of the 90-day feeding experiment. No significant differences in nutrient retention (Table 3) and body composition (Table 4) were observed in all treatments.

Table 2

Growth, feed efficiency and survival rate of juvenile *P. monodon* fed diets containing increasing replacement level of ULM to replace soybean meal

Ulva replacement level	*D1 (0%)*	*D2 (15%)*	*D3 (30%)*
IABW (g)	0.11 ± 0.02	0.11 ± 0.02	0.11 ± 0.02
FABW (g)	1.45 ± 0.12[a]	1.22 ± 0.04[a]	1.03 ± 0.11[a]
FI (g shrimp^{-1})	2.16 ± 0.16[a]	1.70 ± 0.07[a]	1.53 ± 0.24[a]
SGR (% day^{-1})	3.02 ± 1.00[a]	2.82 ± 0.04[ab]	2.61 ± 0.12[b]
FCE	0.67 ± 0.02[a]	0.65 ± 0.01[a]	0.66 ± 0.00[a]
PG	0.20 ± 0.02[a]	0.17 ± 0.01[b]	0.16 ± 0.02[b]
PER	13.12 ± 0.59[a]	14.82± 0.26[a]	14.34± 1.22[a]
Survival rate (%)	87.00 ± 3.85[a]	93 .00± 0.00[a]	87.00 ± 6.70[a]

Values in the same column with different superscript letters are significantly different ($p < 0.05$). Values were expressed as mean ± SEM. IABW - initial average body weight; FABW - final average body weight.

Table 3

Nutrient retention (%) of *P. monodon* fed with diets containing ULM as replacement to soybean meal

Diet	*Protein retention*	*Lipid retention*
Control	22.94 ± 0.98[a]	4.33 ± 0.18[a]
15%	26.75 ± 0.47[a]	4.75 ± 0.08[a]
30%	28.03 ± 2.15[a]	5.01 ± 0.38[a]

Table 4

Body composition (%) of *P. monodon* (dry weight) fed with ULM as replacement of soybean

Diet	*% Crude protein*	*% Crude fat*	*% Crude fiber*	*% Moisture*	*% Ash*	*% NFE*
Initial	55.61	1.90	3.69	6.57	13.67	18.56
Control	59.98	2.92	5.32	4.30	15.79	11.69
15%	60.30	2.88	4.02	5.68	15.57	11.55
30%	61.84	2.98	5.02	4.69	16.12	9.35

Studies on seaweeds as feed ingredient in the diet of cultured species have been done on very few species of fish such as those of Wahbeh (1997), Azaza et al (2008), Ergun et al (2008), Mustafa & Nakagawa (1995) and Wassef et al (2001). Among these studies, only few researches have been done that evaluated seaweed as ingredient in the diet of crustaceans. Seaweed has been evaluated to be a potential ingredient that could be a component in the diets of *Litopenaeus vannamei* (Briggs & Funge-Smith 1996) and *P. monodon* (Da Silva & Barbosa 2009). Incorporating 10% seaweed to the diets of *P. monodon* and *Litopenaeus vannamei* resulted in higher weight gain (5.2% heavier than those fed the control diet), food conversion ratio was lowered by 0.1 point, improved color of shrimp, 25% lower mortality rates, improved taste and texture of the shrimp (Ocean Harvest Technology 2012). The results of the present study were in agreement with the study of Briggs & Funge-Smith (1996) and with that of Ocean Harvest Technogy (2012). Other studies on crustaceans show that *Gracilaria cervicornis* can substitute industrial feeds of *L. vannamei* up to 50% (Marinho-Soriano et al 2007). In the present study, as the level of ULM was increased up to 30% soybean replacement, PER also increased significantly. This indicated that *P. monodon* efficiently utilized protein from the diet containing higher ULM replacement level (30%).

Between the two of groups of shrimp fed diets containing ULM, the better growth and nutrient utilization performance was exhibited by those fed diets at 30% replacement of soybean meal or 10% inclusion rate. ULM can be incorporated in the diets of the gilthead seabream *Sparus auratus* at 4% of the total diet weight (Diler et al 2007), red tilapia *Oreochromis* sp. at 15% (El-Tawil 2010) and common carp *Cyprinus carpio* at 5% inclusion rate (Diler et al 2007) without any adverse effects at least on growth and feed utilization. Shields & Lupatsch (2012) briefly reviewed some of documented benefits of incorporating small amounts in livestock and aquafeeds, which include improved immune system (Turner et al 2002; Declarador & Serrano Jr. 2014; Lauzon & Serrano Jr. 2015); improved lipid metabolism (Nakagawa 1997; Guroy et al 2011); improved gut function (Michiels et al 2012). This is in addition to being a source of protein, amino acids, fatty acids, vitamins and minerals and other biologically active phytochemicals (Pulz & Gross 2004; Becker 2004; Gouveia et al 2008).

It was interesting to compare our previous report on the performance of *U. lactuca* protein concentrate (Serrano Jr. & Santizo 2014) with that of the present study. Growth performance of shrimp fed with 30% soybean replacement (or 10.5% inclusion) *Ulva* protein concentrate (Serrano Jr. & Santizo 2014) and those fed *U. lactuca* protein concentrate (ULPC) were not significantly different (Table 5).

Table 5

Growth performance, feed efficiency, nutrient utilization and survival of juvenile *P. monodon* fed diets containing 30% replacement of soybean with *U. lactuca* protein concentrate (ULPC) from Santizo et al (2014) or with ULM in the present study

Parameters	*30% ULM*	*30% ULPC*
FI (g shrimp^{-1})	1.53 ± 0.24[a]	1.56 ± 0.17[a]
FCE	0.66 ± 0.00[a]	0.65 ± 0.02[a]
PG	0.16 ± 0.02[a]	0.17 ± 0.01[a]
PER	14.34± 1.22[a]	14.60 ± 0.57[a]
Lipid retention (%)	5.01 ± 0.38[a]	5.30 ± 0.22[a]
Survival rate (%)	87.00 ± 6.70[a]	98.00 ± 2.22[a]

Values were expressed as mean ± SEM; Values with the same superscript letters are not significantly different ($p > 0.05$); Paired Student t test was done between the incorporated two forms of *U. lactuca*.

This was despite the observation that the digestibility of the dry matter of ULM was only 71.5% compared to that of the concentrate with 99.1 dry matter digestibility (Santizo et al 2014). We hypothesize that some of the beneficial bioactive compound was lost during the concentration process especially during acid and alkali treatment which included the sulfated polysaccharide ulvan. Ulvan is a component that could improve resistance to pathogens and perhaps degraded environment but also could promote growth

(Declarador & Serrano Jr. 2014; Lauzon & Serrano Jr. 2015). Results of the present study suggested that *U. lactuca* (either UPC or RDUP) successfully replaced soybean in the diet of *P. monodon* up to 30% or 10.5% inclusion rate. Surprisingly, the raw meal was no inferior to that of the protein concentrate regardless of lower dietary protein content of 40.7% in the previous report at replacement level of 30% equivalent to 10.5% inclusion rate viz a viz 37.3% dietary protein in the present study at the same replacement or inclusion rate of 30% (Table 5).

Conclusions. Although the SGR of shrimps fed the diet containing 30% was slightly inferior to those fed the control diet, all the parameters such survival rate, feed intake, food conversion efficiency, protein and lipid retention and body composition were not significantly different from those fed the control diet and those fed diet containing 15% soybean replacement or 5.2% inclusion rate. The performance of diets containing 30% replacement of the soybean meal by ULPC in the previous study and that containing 30% replacement of the ULM in the present study were statistically significant. Incorporating the raw meal was more economical than incorporating the seaweed protein concentrate due to additional processing. It is thus recommended that the raw meal should be used as a replacement of the imported soybean meal in the diet of the shrimp *P. monodon* at 30% replacement level equivalent to 10.5% inclusion rate.

Acknowledgements. The authors wish to express their gratitude to the Philippine Department of Science and Technology (DOST), Philippine Council for Agriculture, Aquatic, and Natural Resources Development (PCAARD) for the funding and scholarship provided. They also wish to thank the UPV Office of the Research and Extension for (OVCRE) for the additional funding and publication support.

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Received: 21 April 2015. Accepted: 18 May 2015. Published online: 19 May 2015.
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How to cite this article:
Serrano Jr. A. E., Santizo R. B., Tumbokon B. L. M., 2015 Potential use of the sea lettuce *Ulva lactuca* replacing soybean meal in the diet of the black tiger shrimp *Penaeus monodon* juvenile. AACL Bioflux 8(3):245-252.