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# Dietary substitution of protein concentrate of *Ulva lactuca* for soybean meal in the black tiger shrimp *Penaeus monodon* fry

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**Abstract**. A feeding trial was conducted to test the protein concentrate of the green seaweed *Ulva lactuca* as a substitute for soybean meal in the diet of the black tiger shrimp *Penaeus monodon* fry. Experimental diets containing various replacement levels for soybean meal by weight were prepared, namely 0 (control diet), 15, 30 or 45 % *Ulva* protein concentrate (UPC). Survival rate was not affected by the diets. Feed intake (FI) was statistically similar in shrimp fed the control diet and diet containing 15 % UPC replacement while the FI of shrimps fed diets containing 30 % and 45 % were lower and were not significantly different from each other. SGR of shrimps fed the control diet was not significantly different from the values of those fed diets containing 15 % and 30 % UPC replacements while those fed diets containing 45 % UPC replacement exhibited significantly the lowest SGR. Protein gained of shrimps were statistically similar between those fed the control diet and those fed diets with 15 % and 30 % UPC while those fed diets at 45 % UPC was significantly lower than the three groups. Protein efficiency ratio (PER) was unaffected by the dietary treatments. *Ulva* protein concentrate could be a substitute for soybean meal in the diet of *Penaeus monodon* fry up to 30 % without compromising the survival, growth and feed utilization efficiency of the shrimp despite lower feed intake.
**Key Words**: Green seaweed, experimental diets, *Ulva* protein concentrate, soybean meal replacement.

**Introduction**. *Ulva* sp. are common in the intertidal zones of the Philippines and sometimes over-proliferate causing blooms or green tides in some protected bays (Largo et al 2004). Exploiting these nuisance species as alternative protein sources in aquafeeds may potentially solve the cost and shortage problems of feed ingredients.

*Ulva* sp. has significant amount of proteins, vitamins, minerals, and growth regulators (Guroy et al 2007). Its crude protein content ranged between 10 % and 26 % of dry weight (Fleurence 1999). *Ulva* meal at 5 % inclusion results in an improved growth performance, feed efficiency, nutrient utilization, and body composition of Nile tilapia (Ergun et al 2009) and enhances growth and pigmentation in shrimp (Sanchez et al 2012).

The production of seaweed meal as dried fine powder has increased its bioavailability for animals (Shelar et al 2012). For economic reasons or for enhancement of the nutritional quality of the food product, the use of plant protein concentrate (PCs) as a functional ingredient is of growing interest in the food industry (Wong & Cheung 2001). During processing of PCs, the non-digestible fiber is eliminated which allows the possible use of higher levels of plant material in fish diets. The application of PCs in aquafeed industry has been limited to protein from legumes (Chatzifotis et al 2006; Forster et al 2002; Olvera-Novoa et al 1997) and very few have been done on seaweeds (Wong et al 2004) specifically red seaweeds *Hypnea charoides* and *Hypnea japonica*.

There have been several studies that have quantified protein from seaweeds (*e.g.* (Fleurence 1999), only a few studies have been undertaken on the nutritional quality of seaweed protein.

This study primarily aims to evaluate levels of inclusion as partial replacements to soybean meal in *Penaeus monodon* diets for growth and nutrient utilization efficiency.

## Material and Method

***Seaweed collection and processing***. *Ulva lactuca* were collected from the shore of Zamboanga State College of Marine Sciences and Technology (ZSCMST). These were then cleaned from debris and washed with freshwater. The seaweed was air-dried as described by Tiroba (2007) where seaweeds were laid flat on the tables under the shaded hut and drying was facilitated by an electric fan. Protein concentrate of the seaweed was prepared following the method of Agbede et al (2008) modified by including an acidification stage. Dried seaweeds were homogenized with distilled water using a hand-held blender, the slurry was acidified by adding HCl to pH 2.0, squeezed through a muslin cloth to collect the juice containing the protein. The juice was heated to 80 – 90 °C for 10 min and the coagulated protein was separated out by filtering through a muslin cloth. The thick protein concentrate slurry was oven-dried to about 10 % moisture and was kept at -20 $^{o}$C until diet preparation.

Table 1

Composition (g kg$^{-1}$ diet) and proximate analysis of diets containing *Ulva* protein concentrate for growth trial experiment

| *Ingredients* | *Dietary treatments (% replacement of soybean meal)* | | | |
|---|---|---|---|---|
| | *0 %* | *15 %* | *30 %* | *45 %* |
| Danish fish meal | 380.0 | 380.0 | 380.0 | 380.0 |
| Squid meal | 29.0 | 29.0 | 29.0 | 29.0 |
| Soybean meal | 350.0 | 298.0 | 245.0 | 193.0 |
| Bread flour | 80.0 | 80.0 | 80.0 | 80.0 |
| Cod liver oil | 63.0 | 63.0 | 63.0 | 63.0 |
| Lecithin | 5.0 | 5.0 | 5.0 | 5.0 |
| CMC | 37.5 | 37.5 | 37.5 | 37.5 |
| Ligno bond | 15.0 | 15.0 | 15.0 | 15.0 |
| [a]Vitamin mix | 10.0 | 10.0 | 10.0 | 10.0 |
| [b]Mineral mix | 10.0 | 10.0 | 10.0 | 10.0 |
| Dicalcium phosphate | 20.0 | 20.0 | 20.0 | 20.0 |
| BHT | 0.5 | 0.5 | 0.5 | 0.5 |
| *Ulva* PC | 0.0 | 52.0 | 105.0 | 158.0 |
| RDUP | 0.0 | 0.0 | 0.0 | 0.0 |
| Total | 1000.0 | 1000.0 | 1000.0 | 1000.0 |
| *Proximate analysis (%)* | | | | |
| Moisture | 4.2 | 4.0 | 4.8 | 4.9 |
| Crude protein | 41.3 | 41.4 | 40.7 | 40.1 |
| Crude fat | 10.8 | 10.7 | 10.4 | 10.2 |
| Crude fiber | 2.5 | 2.0 | 2.9 | 2.9 |
| Ash | 15.3 | 15.1 | 15.9 | 15.4 |
| NFE | 26.0 | 26.9 | 25.3 | 26.4 |
| [c]Gross energy (KJ g$^{-1}$) | 18.4 | 18.5 | 18.0 | 18.0 |

[a] Vitamin mix (mg or IU/kg diet): Vitamin A, 12,000 IU; Vitamin D3, 2,000 IU; Vitamin E, 200 IU; Vitamin $B_1$, 80; Vitamin $B_2$, 80; Vitamin $B_6$, 50$^{1}$; Vitamin $B_{12}$, 2000 mcg kg$^{-1}$; Niacin, 400; Calcium Pantothenate, 200; Biotin, 0.4; Folic Acid, 18 mg kg$^{-1}$; Ethoxyquin, 5.

[b] Mineral mix (mg/kg diet): Fe, 400; Mn, 100; Zn, 400; Cu, 40; I, 18; Co, 0.2; Se, 2.

[c] 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)

***Experimental animal and set up***. The feeding trial was conducted at the Institute of Aquaculture Multispecies Hatchery employing a recirculating system from September to

December 2013. *P. monodon* juveniles (600 individuals) were obtained from the hatchery. Prior studies, samples of shrimp were subjected to the detection of White Spot Syndrome Virus (WSSV) by the Polymerase Chain Reaction (PCR); all the shrimps used in the experiment were disease free. Shrimps were acclimated to the environmental conditions for 2 weeks in a one-ton fiber glass tank equipped with aeration at salinity range of 26 - 28 ppt. The shrimp were fed with control diet 4 times a day (08:00, 11:00, 14:00 and 17:00). Water exchange was done daily at a rate of 10 – 30 %. Shrimp samples were taken, dried and stored in a freezer at -20 °C for initial proximate analysis of carcass.

*P. monodon* juveniles with an average 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 for each tank. The shrimp were further conditioned to the experimental condition for 5 days and fed with the control diet. There were 4 dietary treatments with various replacement levels of soybean meal by UPC in the diet, namely 0 % (*i.e.* the Control diet), 15 %, 30 % and 45 % by weight (Table 2). The experimental diets were fed to shrimp that were randomly assigned to 18 tanks with 3 replicates for each dietary treatment; the feeding trial lasted for 90 days.

***Feeding and management***. Shrimps were fed at the rate of 3 – 15 % average wet body weight divided into 4 equal feedings 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 TAN were maintained at 26.5 - 31.0 °C, 25 - 28 ppt, 7 - 10 mg $L^{-1}$, and 8.0 - 8.5, 0.05 - 0.10 ppm, and 0.10 - 0.20 respectively. Sampling was done every 15 days in which shrimps from each tank were counted and bulk-weighed. Water from the chamber as well as the fiber filter was changed twice a week. To prevent the growth of algae, the experimental tanks were cleaned almost every day. At the end of the experimental period, shrimps in each treatment were pooled, sacrificed and subjected to carcass proximate analysis.

***Growth performance and feed utilization***. Growth performance and feed utilization were evaluated: specific growth rate (SGR), feed conversion efficiency (FCE), protein gained (PG), protein efficiency ratio (PER), survival rate, protein and lipid retention. These parameters were estimated using the following formulae:

$$SGR\ (\%\ day^{-1}) = (\ln FBW - \ln IBW) / D \times 100$$

$$FCE = \text{wet weight gain (g)} / \text{feed consumed (g)}$$

$$PG\ (g) = (\text{final-initial}) / \text{whole body protein}$$

$$PER = \text{wet body weight gain} / \text{protein intake}$$

$$\text{Protein Retention} = [(\%\ \text{final carcass protein} \times \text{final ABW (g)}) - (\%\ \text{initial carcass protein} \times \text{initial ABW (g)}) / \text{total protein intake (g)}] \times 100$$

$$\text{Lipid Retention} = [(\%\ \text{final carcass lipid} \times \text{final ABW (g)}) - (\%\ \text{initial carcass lipid} \times \text{initial ABW (g)}) / \text{total lipid intake (g)}] \times 100$$

Where:

FBW = final body weight

IBW = initial body weight

D = number of days of culture.

***Chemical analysis***. The seaweed protein concentrate, the unprocessed seaweed meal as well as the experimental diets were subjected to proximate analysis, as well as the initial and final carcasses. Moisture was measured using a thermo-balance (Mettler 32 Toledo HB43 halogen moisture analyzer). Ash content was determined after incineration in a muffle furnace at 550 $^{o}$C for 12 h (AOAC 1990). Crude protein was measured after block digestion and steam distillation using Foss Tecator™ digestion system and Foss Kjeltec™ 8200 auto-distillation unit. Crude fat was extracted using Foss Soxtec™ 2050 automatic system and fiber was determined using Foss Fibertec™ 2010 system.

***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 dietary treatment. Data were analyzed for normal distribution using Kolmogorov-Smirnov test and Levene's test for homogeneity of variances. Data on 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. Difference was regarded as significant when $P<0.05$.

**Results**. All shrimps readily accepted the feeds and fed normally in the entire period of the experiment.

Table 2

Proximate composition of unprocessed *Ulva* meal and *Ulva* protein concentrate (UPC) (% d.m.)

| *Composition (DM, %)* | *Ulva lactuca Protein Concentrate* | *Unprocessed Ulva lactuca meal* |
|---|---|---|
| Moisture | 12.5 | 14.5 |
| Crude protein | 38.4 | 13.4 |
| Crude lipid | 1.06 | 0.88 |
| Crude fiber | 2.8 | 4.4 |
| Ash | 14.7 | 31.7 |

Table 3

Growth, feed efficiency and survival of juvenile *Penaeus monodon* fed diets containing increasing replacement level of *Ulva* protein concentrate (UPC) to replace soybean meal

| *Parameter* | *% Ulva* | | | |
|---|---|---|---|---|
| | *0 %* | *15 %* | *30 %* | *45 %* |
| Survival (%) | 87 ± 3.85$^{a}$ | 96 ± 2.22$^{a}$ | 98 ± 2.22$^{a}$ | 91 ± 5.88$^{a}$ |
| IABW (g) | 0.11 ± 0.02 | 0.11 ± 0.02 | 0.11 ± 0.02 | 0.11 ± 0.02 |
| FABW (g) | 1.45 ± 0.12$^{a}$ | 1.23 ± 0.03$^{ab}$ | 1.16 ± 0.10$^{ab}$ | 0.98 ± 0.01$^{b}$ |
| FI (g shrimp-1) | 2.16 ± 0.16$^{a}$ | 1.66 ± 0.04$^{ab}$ | 1.56 ± 0.17$^{b}$ | 1.43 ± 0.01$^{b}$ |
| SGR (% day-1) | 3.02 ± 1.00$^{a}$ | 2.84 ± 0.32$^{ab}$ | 2.76 ± 1.00$^{ab}$ | 2.57 ± 0.01$^{b}$ |
| FCE | 0.67 ± 0.02$^{a}$ | 0.66 ± 0.02$^{a}$ | 0.65 ± 0.02$^{a}$ | 0.63 ± 0.02$^{a}$ |
| PER | 13.12 ± 0.59$^{a}$ | 14.33 ± 0.17$^{a}$ | 14.60 ± 0.57$^{a}$ | 13.45 ± 1.10$^{a}$ |
| PG | 0.20 ± 0.02$^{a}$ | 0.19 ± 0.01$^{ab}$ | 0.17 ± 0.01$^{ab}$ | 0.15 ± 0.00$^{b}$ |

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; FI - feed intake; SGR - specific growth rate; FCE - feed conversion efficiency; PG - protein gained; PER - protein efficiency ratio.

Survival rate was not affected by the diets during the entire period of the experiment (Table 3). Final average body weight (FABW) was statistically similar in shrimp fed the control, 15 % and 30 % UPC replacements while those fed diets with 45 % UPC replacement was significantly the lowest among the experimental groups.

Feed intake (FI) were statistically similar in shrimp fed the control diet and diet containing 15 % UPC replacement while the FI of shrimps fed diets containing 30 % and 45 % were lower and were not significantly different from each other.

SGR of shrimps fed the control diet was not significantly different from the values of those fed diets containing 15 % and 30 % UPC replacements while those fed diets containing 45 % UPC replacement exhibited significantly the lowest SGR.

Protein gained of shrimps were statistically similar between those fed diet the control diet and those fed diets with 15 % and 30 % UPC replacements while those fed with diets at 45 % UPC replacement was significantly lower than the three groups. In contrast, protein efficiency ratio (PER) was unaffected by the dietary treatments.

Table 4

Nutrient retention (%) of *Penaeus monodon* fed with UPC as a substitute for soybean meal

| *Group* | *Protein retention* | *Lipid retention* |
|---|---|---|
| Control | 22.94 ± 0.98[a] | 4.33 ± 0.18[b] |
| 15% | 27.86 ± 0.30[a] | 6.04 ± 0.06[a] |
| 30% | 26.89 ± 1.09[a] | 5.30 ± 0.22[ab] |
| 45% | 26.45 ± 2.18[a] | 5.11 ± 0.42[ab] |

Values in the same column with different superscript letters are significantly different ($P < 0.05$).

Protein retention was not affected by the experimental diets (Table 4). In contrast, lipid retention was significantly the highest in shrimps fed the diet containing 15 % UPC but this was statistically similar in shrimps fed diets containing UPC while those fed the control diet was significantly the lowest but was not significantly different from those shrimps fed diets containing 30 % and 45 % UPC.

Table 5

Body composition (%) of *Penaeus monodon* (dry weight) fed with UPC as replacement of soybean

| *Specification* | *Moisture* | *Crude protein* | *Crude fat* | *Ash* |
|---|---|---|---|---|
| Initial | 6.57 | 55.61 | 1.90 | 13.67 |
| Control | 4.30 | 59.98 | 2.92 | 15.79 |
| 15% | 5.89 | 61.86 | 3.41 | 15.55 |
| 30% | 4.33 | 62.14 | 3.10 | 15.88 |
| 45% | 4.30 | 62.93 | 3.06 | 15.92 |

Incorporating UPC in the diet of shrimps increased the carcass protein and crude fat while body ash seemed to remain similar (Table 5).

**Discussion**. Only the dietary treatment with 45 % UPC replacement level was inferior to the growth performance of shrimp fed the control diet in terms of final ABW and SGR. Although UPC exhibits high dry matter digestibility as was shown previously (Santizo et al 2014), this reduced ABW and SGR can be due to the reduced feed intake (FI) of the shrimp, suggesting reduced palatability at 45 % soybean replacement level. At soybean replacement levels of 15 % and 30 %, most of the indices were statistically similar with those fed the control diet except feed intake as summarized in Table 3. In rainbow trout, complete substitution of fish meal with soy protein concentrate (SPC) resulted in either reduced growth (Medale et al 1998; Stickney et al 1996) or no negative effects (Kaushik et al 1995). The reduced growth observed in other fish was assumed to be due to lower feed intake as a result of reduced palatability (Davis et al 1995; Stickney et al 1996). In the present study, feed intake of shrimps fed with diets containing 30 % and 45 % UPC were statistically lower than those fed the control diet. This finding was similar to that of the Nile tilapia fry fed with diets containing 30 % and 45 % *Ulva intestinalis* protein concentrate (Serrano & Aquino 2014). This could be a function of antinutrients present in small amounts in Ulvales such as saponins, tannins and phytic acid as reported by Azaza et al (2008) in *Ulva rigida*, although dietary saponins could have positive effects on feed intake and efficiency as reported in the common carp *Cyprinus carpio* (Serrano 2013). Feed intake of the Nile tilapia is high at 30 % soybean replacement level of unprocessed

*U. rigida*, Azaza et al (2008) have explained that this is due to the lower digestible energy of the seaweed meal which induces higher feed intake. This was not observed in *P. monodon* fry in the present study considering that the apparent dry matter digestibility of *U. lactuca* as feed ingredient was high (99.13 %) for *P. monodon* (Santizo et al 2014). Despite the reduced feed intake of shrimp at 30 % replacement of UPC, indices for growth and feed utilization efficiency in the present study indicated the possibility of the dietary substitution of 30 % by weight of UPC for soybean meal in the diet of the black tiger shrimp.

A number of studies on the inclusion of unprocessed seaweed meal in the diet of shrimps have been conducted. Results may vary with shrimp species, the type of algae and inclusion level. *P. monodon* fry (200 mg) exhibited better weight gain when fed 5 % *Kappaphycus alvarezii* but a lower growth when fed 3 % *Gracilaria heteroclada*, these differences disappeared when bigger shrimps were fed with either macroalgae (Peñaflorida & Golez 1996). Unprocessed seaweed meal made from cultivated *Ulva clathrata* at 3.3 % in the diet of *Litopenaeus vannamei* (1.6 g) resulted in better growth than did the wild brown algae *Macrocystis pyrifera* or *Ascophyllum nodosum* for 28 days (Cruz-Suarez et al 2009). In the present study, the nutrient and feed utilization efficiency of shrimps fed diets with UPC at 15 % soybean replacement level in the present study were statistically similar with those of shrimps fed the control diet. In *P. monodon* fry, *Ulva* protein concentrate (UPC) could replace soybean meal in the diets for *P. monodon* up to 30 % (equivalent to 10.5 % inclusion level in the diet) without compromising its growth. This is in agreement with the findings of Briggs & Funge-Smith (1996) in which *Gracilaria* meal can replace soybean meal up to 10 % inclusion in the diet of *P. monodon*. Similarly, Carter & Hauler (2000) have demonstrated that pea and lupin protein concentrate improve growth of Atlantic salmon when fed with 27 % and 22 % replacement of fishmeal, respectively.

Protein retention was similar for all dietary treatments and this could either be an indication that amino acid profile of the UPC was not totally inferior to that of the control diet or that the small proportion of the replaced soybean meal did not have considerable effect on the overall balance of essential to nonessential amino acids *i.e*. the quality of protein. Kumar & Kaladharan (2007) have quantified and characterized the amino acid profile of *U. lactuca*. The amino score of *U. lactuca* was 0.7 and is generally rich in aromatic amino acids, threonine and tryptophan and deficient in sulphur containing amino acids, leucine and lysine. Thus, it could serve as a protein source that could complement other protein sources such as soybean meal.

**Conclusions**. *Ulva* protein concentrate could be a substitute for soybean meal in the diet of *P. monodon* fry up to 30 % without compromising the survival, feed intake, growth and protein retention of the animal. Beyond 30 % soybean replacement level, feed intake was significantly reduced suggesting reduced palatability while at 45 % soybean replacement level, growth was reduced.

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