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Growth performance of black tiger shrimp *Penaeus monodon* larvae fed diets supplemented with ulvan

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Abstract. Previously, we have demonstrated the efficacy of ulvan extracted from *Enteromorpha intestinalis* as immunostimulant in *Penaeus monodon* and *Litopenaeus vannamei*. The present study aimed to investigate whether or not ulvan could enhance the growth performance of *P. monodon* larvae. Experimental diets were formulated to contain four levels of isolated ulvan, namely 0.0, 500 mg, 1,000 mg, and 1,500 mg kg^{-1} and were fed to 5 replicates of shrimp larvae for 56 days. Total feed intake, specific growth rate and survival of shrimps fed ulvan-supplemented diets were all statistically similar with those fed diets with no supplementation (i.e. the control diet). Significant differences were detected in feed conversion efficiency of shrimp fed the dietary treatments but the enhancement by the ulvan-supplemented diet was not very clear. In contrast, protein gained (i.e. protein productive value, PPV) of shrimps fed 1,000 mg and 1,500 mg ulvan kg^{-1} diet elicited significantly higher protein productive value (PPV) than did shrimps fed the control diets, the latter dose exhibiting significantly the highest PPV. In conclusion, ulvan supplementation resulted in higher protein gain which was the benefit of dietary ulvan at least at the level of 1,000 mg kg^{-1} in *P. monodon* larvae.
Key Words: Protein productive value, sulfated polysaccharides, specific growth rate, feed intake, survival rate.

Introduction. Previously, we have shown that supplementing ulvan at 1,000 mg kg^{-1} (i.e. 0.1 %) to the diet of *Penaeus monodon* prolonged survival than did those fed diets without ulvan supplementation (Declarador et al 2014). Furthermore, they exhibited higher total hemocyte count (THC), higher respiratory burst activity and higher phenoloxidase activity which indicated activation of cellular immunity in shrimps. Adding ulvan at 1,000 to 1,500 mg kg^{-1} diet (0.1 – 0.15 %) might have stimulated hemocytic degranulation and activated prophenoloxidase to become phenoloxidase. We also have shown the comparative effects of ulvan on immunomodulatory activities of *Litopenaeus vannamei* and *P. monodon* (unpublished data). In both penaeid shrimps, ulvan supplementation resulted in increased THC, respiratory burst and phenoloxidase activities. The optimum dietary ulvan levels that elicited the maximum responses in the immune responses (I*max*) of *L. vannamei* and *P. monodon* were 0.21 % and 0.15 %, respectively.

Ulvans are structural acid polysaccharides present in cell wall of green algae (*Ulva* and *Enteromorpha*). They are highly sulphated and essentially consists of rhamnose 3-sulfate, xylose, xylose 2-sulfate, glucuronic acid and iduronic acide units. These monomers are arranged in an essentially linear fashion even though a slight degree of branching has been found (Lahaye & Robic 2007). Ulvan has a wide list of beneficial biological effects reported by the literature span from antioxidant (Qi et al 2006) to anticoagulant (Zhang et al 2008), antitumoral (Kaeffer et al 1999) antihyperlipidemic (Yu et al 2003) and immunomodulating (Leiro et al 2007) activities, proved both *in vitro* and *in vivo*. The structure of the repeating unit in ulvan resembles that typical of glycosaminoglycan which represent the trigger of the immunomodulating activity (Chiellini & Morelli 2011).

Compounds that act as immunostimulants in aquaculture species sometimes are also growth or conversion efficiency promoters. For example, various herbal products such as *Hygrophila spinosa* (marsh barbel) *Withania somnifera* (Indian ginseng), *Zingiber officinale* (ginger), *Solanum trilobatum* (purple fruited pea eggplant), *Andrographis paniculata* (king of bitters), *Psoralea corylifolia* (Psoralea seeds), *Eclipta erecta* (eclipta), *Ocimum sanctum* (holy basil), *Picrorhiza kurroa* (picrorhiza), *Phyllanthus niruri* (stone-breaker), *Tinospora cordifolia* (heart-leaved moonseed) have the characteristics of growth promotion, anti-stress, immunostimulation, and antibacterial. These preparations had a good influence in the *Penaeus* larviculture (Citarasu et al 1998, 2002).

The present study aimed to evaluate the growth performance of *P. monodon* fry fed diets with the commercial ulvan extracted from *Enteromorpha intesnalis*.

Material and Method

Experimental animals. *Penaeus monodon* post-larvae (19 days after hatch or PL 19) were obtained from the Southeast Asian Development Center - Aquaculture Department (SEAFDEC-AQD) in Tigbauan Iloilo, Philippines. They were maintained and reared in a 5-ton capacity tank at the University of the Philippines Visayas Multi-Species Hatchery. The shrimps were acclimated and fed with commercial shrimp diet for 2 weeks. Prior to the experiment, the shrimps were randomly selected and screened by one-step PCR for WSSV infection and found to be free of the virus.

Experimental set-up. Growth trial was conducted using a recirculating system employing biological and mechanical filtrations e.g. pebbles and fiber fill (Figure 1). Water was recirculated from the chamber to the 50 L capacity aquaria at a flow rate of approximately 600 mL min^{-1} $tank^{-1}$. Water quality parameters, such as salinity (25 - 27 ppt), temperature (25 – 27 °C), pH (8.5 - 9.0) and dissolved oxygen (>5 ppm) were monitored weekly. Nitrite (0 - 0.015 ppm) and total ammonia-nitrogen (TAN) (0 - 0.02) were measured using commercially available kits (CP Aqua Test kits), and maintained at a low level by 100 % water change in the chamber every 5 - 7 days. Each aquarium was provided with adequate aeration and cleaned daily by siphoning uneaten feeds and faeces before feeding.

Figure 1. Recirculating system used in the feeding trial made up of twenty 50 L aquaria and biological and mechanical filtration.

Test diets. Experimental diets were formulated to contain four levels of native grade ulvan (Elicityl, France) namely 0.0, 500 mg, 1000 mg, and 1500 mg kg^{-1} and were fed to 5 replicates of shrimp larvae for 56 days.

Diets were prepared by mixing all dry ingredients with fish oil followed by the addition of isolated ulvan at 0.0 mg (Diet A), 500 mg (Diet B), 1,000 mg (Diet C), and

1,500 mg kg^{-1} (Diet D) of feed (Table 1) dissolved in hot water by sonication (5 g L^{-1}). Addition of isolated ulvan in feed was made by adjusting the amount of cellulose. Cooked starch was added and mixed until it resulted in moist dough. The resulting dough was passed through the pelletizer twice to ensure proper mixing and was steamed for 5 min and oven-dried at 60 °C for 18 - 24 h. The pellets were cut into appropriate sizes and stored at 4 °C until use. Table 1 shows the proximate composition of the diets.

Table 1

Composition and proximate analysis of experimental diets containing various levels of native grade ulvan

Ingredients	*Diet A (0.0 mg ulvan)*	*Diet B (500 mg ulvan)*	*Diet C (1000 mg ulvan)*	*Diet D (1500 mg ulvan)*
Peruvian fish meal	20.00	20.00	20.00	20.00
Shrimp meal	34.00	34.00	34.00	34.00
Soybean meal	21.00	21.00	21.00	21.00
CMC	3.48	3.43	3.38	3.33
Vitamin mix	1.00	1.00	1.00	1.00
Mineral mix	1.00	1.00	1.00	1.00
BHT	0.02	0.02	0.02	0.02
Lecithin	0.50	0.50	0.50	0.50
Cod liver oil	4.00	4.00	4.00	4.00
Starch	15.00	15.00	15.00	15.00
Purified ulvan	0.00	0.05	0.10	0.15
Total	100.00	100.00	100.00	100.00
Proximate composition (%)				
Crude protein	40.56	38.77	38.47	38.30
Moisture	8.67	9.99	9.36	9.43
Crude fat	7.38	7.47	6.82	7.56
Ash	13.63	14.35	14.39	14.83
Fiber	3.59	3.70	4.02	3.64
NFE	26.17	25.72	26.94	26.24
Total	100.00	100.00	100.00	100.00

Feeding trial. Shrimps post larvae (average body weight, ABW of 0.013 g) were stocked into twenty 50 L aquaria with 25 shrimps in each aquarium and were acclimatized to experimental condition for 3 days. Three experimental and control diets were fed to shrimps in five replicates in a completely randomized design.

Feeding was done thrice daily (08:00, 12:00 and 16:00) for 56 days at a ration starting at 20 % of average body weight and decreased to 10 % towards the end of the experimental period. Shrimps were bulk-weighed every 14 days and the amount of feed to be given the following week was estimated. Growth, survival and nutrient utilization were quantified using the following formulas.

$$\text{Specific growth rate (SGR, \% day}^{-1}) = (\ln W_f - \ln W_i) \times 100 / \text{days of culture}$$

$$\text{Feed conversion efficiency (FCE, \%)} = (W_f - W_i) \times 100 / \text{feed given}$$

$$\text{Protein productive value (PPV, \%)} = (CP_f \times W_f) - (CP_i \times W_i) \times 100 / \text{protein fed}$$

Where:

W_i- initial ABW

W_f- final ABW

CP_i- initial carcass protein

CP_f- final carcass protein.

Statistical Analysis. Data obtained from the feeding trial such as total feed intake (TFI), specific growth rate (SGR), survival rate, feed conversion efficiency (FCE) and protein productive value (PPV) were tested for normality of distribution using Shapiro-wilk test and homogeneity of variance using Levenes test. When data did not pass these two tests, they were transformed until they pass the tests and data were analyzed using one-way analysis of variance (ANOVA). When significant differences between treatments means were detected, they were ranked by post-hoc analysis (Tukey's test). All probability values were set at a significance level of 0.05.

Results and Discussion

Feed intake. Total feed intake (TFI) of the experimental groups were not significantly different from each other which meant that diet supplemented with ulvan was as palatable as that with none (i.e. control diet, Table 2). It has been reported that some compounds from seaweed extracts such as amino acids, digalactosy-diacylglycerol , 6-sulfoquinovolsyldiacylglycerol, phosphatidylethanolamine and phosphatidylcholine can act as attractants in pelleted diets for abalone (Sakata & Ina 1985; Sakata et al 1991). *Ulva lactuca* has been shown to contain digalactosyldiacylglycerol (Kumari et al 2013). Green algae, particularly the Ulvales, are a good source of dimethyl sulfonyl propionate (DMSP) (Van Alstyne et al 2001) which has been shown to act as an attractant in shrimp (Liang et al 2001).

Table 2

Specific growth rate and survival of *Penaeus monodon* fry fed various diets containing ulvan

Parameter	*0.00 mg ulvan*	*500 mg ulvan*	*1000 mg ulvan*	*1500 mg ulvan*	*P*
TFI	0.30[a]	0.30[a]	0.29[a]	0.34[a]	NS*
SGR	4.60 ± 0.15[a]	4.47 ± 0.16[a]	4.65 ± 0.13[a]	4.76 ± 0.07[a]	NS*
Survival (%)	93.6 ± 2.0[a]	96.0 ± 2.5[a]	96.8 ± 2.3[a]	93.6 ± 2.4[a]	NS*

TFI = Total feed intake (g), SGR = specific growth rate (% day^{-1}), *NS = no significant difference (P>0.05).

Growth and survival rates. Specific growth rates of shrimps fed ulvan-supplemented diets were high and were not statistically different from those fed the control diet (Table 2) as indicated by the observed frequency of molting with subsequent change in size of the shrimp during the 56-day feeding trial in the present study. Survival rates observed were excellent (93 – 98 %) and were statistically similar in all treatments (Table 1). There also have been studies that have shown no significant differences in diets supplemented with sulfated polysaccharides with those that were not. For example, when 2 g kg^{-1} diet of fucoidan from *Fucus vesiculosus* or κ-carrageenan from *Eucheuma cottonii* is incorporated into the diet and fed to *P. monodon* fry, it has resulted in statistically similar growth with those fed the control diet (Traifalgar et al 2013). Another aspect is the growth performance of *L. vannamei* which was not significantly different from the values of shrimps fed a commercial diet when 50 % of the ration was replaced by *Gracilaria* (Marinho-Soriano et al 2007). In contrast, shrimps fed diets supplemented also with sulphated polysaccharides result in better growth rates. For example, alginic acid from the brown algae *Laminaria digitata* elicits better growth response in *L. vannamei* (Montero-Rocha et al 2006). In small shrimp *P. monodon* (200 mg), 5 % *Kappaphycus alvarezii* meal resulted in the best weight gain and the lowest growth with the supplementation of 3 % *Gracilaria heteroclada* (Peñaflorida & Golez 1996). One possible reason that growth rates are improved in some shrimps is because sulfated polysaccharides interact with a variety of sulfated polysaccharides-binding proteins, such as growth factors (Chen et al 2008). Polysaccharides can also act as prebiotics (i.e. substances that stimulate the growth of beneficial bacteria in the digestive tract) and

exert growth-promoting and health-improving effects (Chojnacka et al 2012). Most of the positive health effects induced by ulvan as a sulfated polysaccharide are generated by the presence of sulphate groups in its structure (Wijesekara et al 2011).

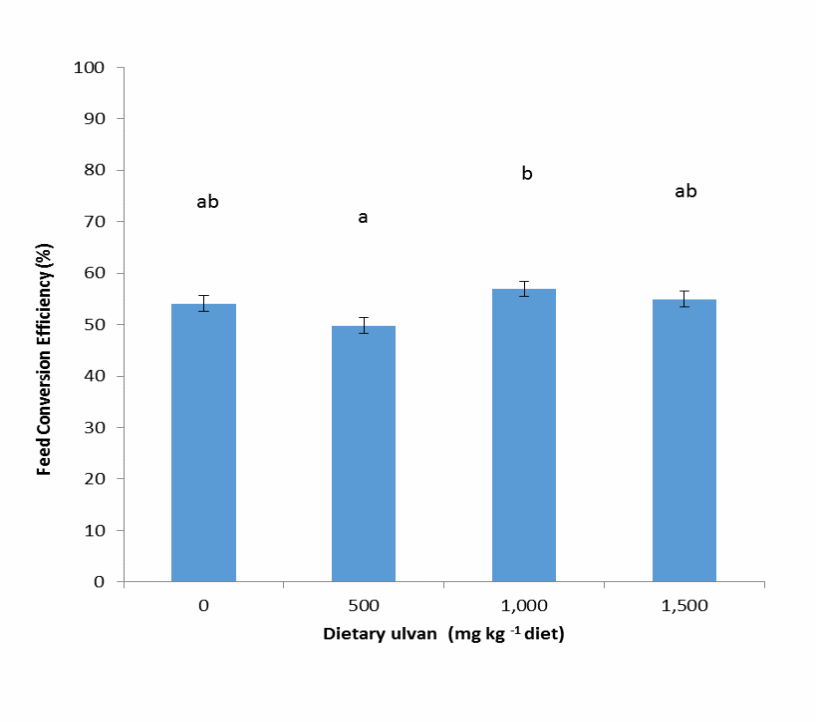

Figure 2. Feed conversion efficiency (FCE) of shrimp fed with various levels of isolated ulvan for 56 days. Values expressed as Mean ± SEM of 5 replicates per treatment.

Feed conversion efficiency (FCE) and protein productive value (PPV). Results for the FCE in the present study although showed significant differences were, ultimately did not indicate in a clear fashion the advantage of incorporating ulvan in the diet (Figure 2). This was in contrast to a number of findings on feeding seaweed-supplemented diet to shrimps. When 3.3 % *Ulva clathrata*, kelps or meal and *Ascophyllum nodosum* are incorporated in the diet and fed to *L. vannamei* juvenile (1.6 g), no significant differences in feed consumption and survival are found, similar to the results of the present study, but the *Ulva* diet results in a better feed conversion ratio (Cruz-Suarez et al 2009). Feed conversion ratio in *P. monodon* was 14 % lower (i.e. better FCE) in a diet with 10 % *G. heteroclada* (Peñaflorida & Golez 1996). The FCR obtained with *U. clathrata* meal was significantly lower (i.e. higher FCE) than that obtained with *Macrocystis* and *Ascophyllum* (Cruz-Suarez et al 2008).

Shrimps fed with diets supplemented with 1,000 mg and 1,500 mg ulvan kg^{-1} diet in the present study elicited significantly higher protein productive value (PPV) than did shrimps fed the control diets (Figure 3) with the latter dose exhibiting significantly the highest PPV among treatment groups. One possible explanation was that algae can increase absorption and assimilation of dietary protein (Yone et al 1986a, 1986b) and can modulate lipid metabolism (Nakagawa et al 1984, 1987, 1997). On the other hand, like the growth promoters, ulvan helps to induce transcription rate thus increasing RNA, total amino acids and finally increases production of protein in the cells (Citarasu 2010). Other sulfated polysaccharides from various seaweeds result in improved protein retention in fish or shrimp. For example, shrimp fed *Ulva* diet exhibited higher protein efficiency ratio (PER) compared with shrimp fed diets containing *Macrocystis* and *Ascophyllum* meals (Cruz-Suarez et al 2008). In snakehead fry (Hashim & Mat-Saat 1992), abalone (Viera et al 2005) and rohu (Bindu & Sobha 2004) improved PER have been observed with the inclusion of pure carrageenan, *Ulva* meal, *Hypnea spinella*, *Gracilaria* cornea, *Ulva fasciata*, *Spyridia insignis* and *Sargassum wightii*. In contrast, PER

is unchanged in sea bass when fed diet supplemented with *Gracilaria bursa-pastoris*, *Ulva rigida*, *G. cornea* and *Ascophyllum* (Nakagawa et al 1997; Valente et al 2006).

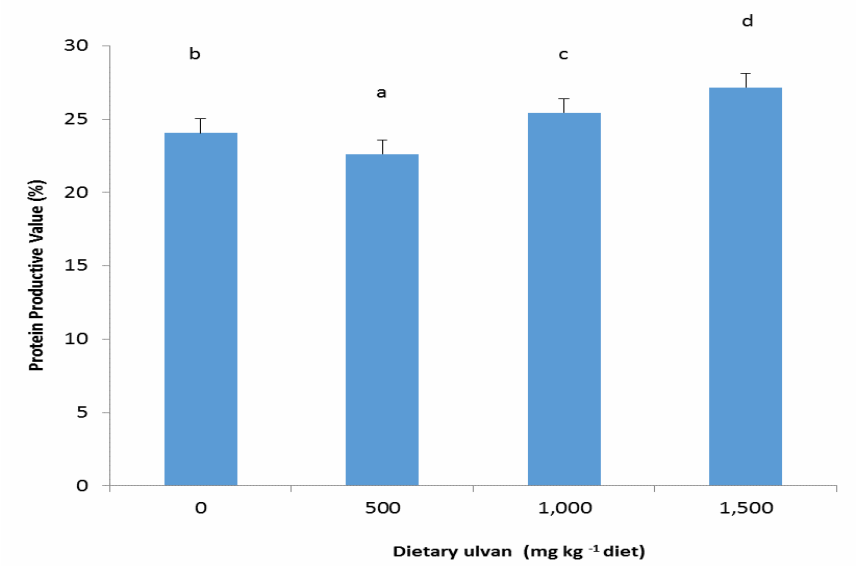

Figure 3. Protein productive value (%) of shrimp fed with different levels of purified ulvan for 56 days. Values expressed as Mean ± SEM of 5 replicates per treatment.

Conclusions. Feeding diets supplemented with ulvan from *Enteromorpha intestinalis* resulted in similar total feed intake, specific growth rate and survival and somewhat statistically similar food conversion efficiency with the diet with no supplementation (i.e. the control diet). However, it significantly increased protein gain in shrimps compared to those fed the diet without ulvan supplementation when the dietary level was at least 1,000 mg kg^{-1} diet.

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