

## Evaluation of dietary supplementation of aqueous extract of brown algae *Sargassum cristaefolium* on growth performance and feed utilization of juvenile white shrimp *Litopenaeus vannamei*

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**Abstract.** A 42-day indoor feeding trial was conducted to evaluate the growth performance and feed utilization of juvenile white shrimp *Litopenaeus vannamei* fed with diets containing different supplement levels of hot-water extract of brown tropical macro algae *Sargassum cristaefolium* extract. The commercial white shrimp feeds containing 36% crude protein were incorporated with graded levels of brown algae *S. cristaefolium* extract (0, 200, 600, 1000 and 1400 mg kg<sup>-1</sup>) and used in the feeding trial. Shrimp (mean initial weight, 2.65±0.11 g) were fed three times daily *ad libitum* at an initial feeding allowance of 8% total body weight day<sup>-1</sup>. A completely randomized design was used in the study and shrimp were stocked at a density of 10 animals 30 L<sup>-1</sup> black round plastic tank in triplicates. Results showed that the different levels of dietary brown algae extract supplement did not significantly affect ( $p > 0.05$ ) on final body weight (10.6-11.3 g shrimp<sup>-1</sup>), survival (80-96.7%), and total moulting shrimp (36-48 times). In terms of weight gain and average daily growth (ADG), the diets with no algae extract supplement and the highest extract inclusion level of 1400 mg kg<sup>-1</sup> diet seemed to have better performance ( $p < 0.05$ ) than other diets. However, the shrimp fed diets containing *S. cristaefolium* extract at 200, 600 and 1000 mg kg<sup>-1</sup> diet showed a better feed utilization (Feed Conversion Ratio (FCR) 1.8-2.0; Protein Efficiency Ratio (PER) 1.40-1.56) than those of shrimp fed the algae extract at levels of 0 mg and 1400 mg kg<sup>-1</sup> diet (FCR 2.38-2.47; PER 1.13-1.17). The results indicated that dietary *S. cristaefolium* extract supplementation had a significant influence ( $p < 0.05$ ) on feed utilization. Shrimp fed the 200-1000 mg algae extract kg<sup>-1</sup> diet had a significantly better FCR and PER ( $p < 0.05$ ) than the shrimp fed the 0 mg and 1400 mg algae extract. These results suggest that supplementation of *S. cristaefolium* extract at a dose of 200-1000 mg kg<sup>-1</sup> can be used to get a better feed utilization performance (reduce 22% FCR and enhance 27.8% PER) of juvenile white shrimp *L. vannamei*. However, a dose of 600 mg *S. cristaefolium* extract kg<sup>-1</sup> diet is recommended to add in diet to get a better FCR and PER performance of juvenile *L. vannamei*.

**Key Words:** *Sargassum cristaefolium*, *Litopenaeus vannamei*, weight gain, feed intake, survival.

**Introduction.** During the past decades, global shrimp farming has suffered severe economic losses because of disease outbreaks mainly caused by fungi, bacteria and virus (Vaseeharan & Ramasamy 2003). Therefore, prevention and control of diseases using probiotics such as lactic acid bacteria and *Bacillus* spp. are very important (Tung et al 2009). Low attention on aquaculture environment management practice and feed quality will be a trigger to the emergence of disease causing the fail harvest of shrimp in the brackishwater pond. It was reported that feeds with addition of immunostimulant can enhance growth, survival, and immunity of some penaeid shrimp such as *Fenneropenaeus chinensis* (Huang et al 2006), *Litopenaeus vannamei* (Yeh et al 2006; Huynh et al 2011; Sirirustananun et al 2011; Chang et al 2013; Kitikiew et al 2013), *Marsupenaeus japonicus* (Traifalgar et al 2010), and *Penaeus monodon* (Felix et al 2004; Traifalgar et al 2009; Immanuel et al 2010).

Bioactives of polysaccharide immunostimulant extracted from macro algae such as caragenan, laminaran, alginate and fucoidan have been reported to be effective to

enhance the immune system of penaeid shrimp (Chotigeat et al 2004; Cheng et al 2005; Yeh & Chen 2008; Huynh et al 2011; Chang et al 2013; Kitikiew et al 2013). Dietary supplementation of immunostimulant (alginate and fucoidan) extracted from brown algae (*Sargassum polycystum*) has been documented to improve immune response and resistance of juvenile shrimp *Marsupenaeus japonicus* (Traifalgar et al 2010), *P. monodon* (Chotigeat et al 2004; Immanuel et al 2010), *Litopenaeus vannamei* (Cheng et al 2005; Chang et al 2013) and *Fenneropenaeus indicus* (Ghaednia et al 2011) against bacterial and viral infections. However, in contrast to dietary administration of immunostimulants ( $\beta$ -glucan, peptidoglycan, polisaccharides, alginate and fucoidan), which effects on shrimp immune response and physiology are well-established, information regarding the effects of polysaccharides extracted from brown algae *Sargassum cristaefolium* on juvenile penaeid shrimp *L. vannamei* growth and feed utilization performances has not been available to date.

The present work was undertaken to evaluate the effects of dietary supplementation of hot-water extract of tropical brown algae, *S. cristaefolium*, on growth, feed utilization and survival performances of juvenile white shrimp *L. vannamei* cultured under laboratory conditions.

**Material and Method.** The study was conducted in June-July 2014 at the Feed and Nutrition Laboratory, Brackishwater Aquaculture Research Centre, Jepara, Central Java Province, Indonesia.

**Preparation of the brown algae extract.** Hot-water extract of brown algae *S. cristaefolium* extract was prepared in the laboratory based on the method of Fujiki et al (1992) and Dub & Dugani (2013). *S. cristaefolium* were collected from around the coastal water of Bandengan District, Jepara Regency, Central Java Province, Indonesia. The algae were cleaned from extraneous matter and properly washed with fresh water, left to dry naturally overnight on plastic net at room temperature, then cut and crushed into small pieces. The crushed algae, then were dried in hot-air-oven at 40°C for 24 h. The dried algae were finely ground using an electric grinder and passed through a 250  $\mu$ m mesh sieve. A hundred g of the milled algae was added to 3000 mL of deionized water, and then the suspension was boiled for 3 h. The suspension was filtered through a nylon mesh, and the filtrated extract was kept. The residue was boiled again with the water 2000 mL for 2 h. The second filtrated extract and the first one were mixed and then gradually evaporated using the pan heated on the stove until it becomes thick to form a pasta. The approximate chemical compositions of hot-water extract of the algae were determined by AOAC method (1998). The compositions were moisture 32.2%, crude protein 1.55%, crude fat 0.61%, crude ash 47.18% and total carbohydrate 20.45%.

**Experimental diets.** Commercial feeds (Starter I Pellets 933S Gold Coin) for *L. vannamei* with 36% crude protein content obtained from suppliers in Jepara, Central Java Province were used as basal diets in the study. In preparation to make experimental diets, the commercial feeds were ground to small particle sizes in a laboratory using an electric grinder and then sieved through a 250  $\mu$ m sieve. All feed materials according to the formulation in Table 1 (except the hot-water extract) was thoroughly mixed in a commercial food mixer for 15 min. Sufficient volume of water (30%) was slowly added to make a stiff dough. The wet mixture was steamed for 5 min and the diets were produced in a noodle-like shape of 2.0 mm in diameter using a meat grinder. Then the pelleted diets were dried overnight at 55°C. After drying, the diets were broken up, sieved into appropriate pellet sizes, packed in plastic bags and stored in a freezer until used for feeding trials. Five isonitrogenous experimental diets containing approximately 36% crude protein (dry weight basis) were formulated to contain 0, 200, 600, 1000 and 1400 mg hot-water extract  $\text{kg}^{-1}$  diet (Table 1).

Table 1

Composition (g kg<sup>-1</sup>) of experimental diets for juvenile *L. vannamei*

Ingredient	Composition (g 1000 g <sup>-1</sup> )				
	Diet A	Diet B	Diet C	Diet D	Diet E
<i>S. cristaefolium</i> extract (100-700 mg L <sup>-1</sup> )	-	0.2	0.6	1.0	1.4
CMC (binder) (0.5%)	5.0	5.0	5.0	5.0	5.0
Cr <sub>2</sub> O <sub>3</sub> (innert marker; 1%)	10.0	10.0	10.0	10.0	10.0
Basal diet* (98.5%)	985.0	984.8	984.4	984.0	983.6
Total (100%)	1000.0	1000.0	1000.0	1000.0	1000.0
<i>Proximate analyses (% dry basis)**</i>					
Crude protein (36%)	355.8	372.9	360.0	366.4	364.2
Crude fat (8-9%)	86.4	87.5	89.9	78.6	90.1
Crude ash (11-12%)	117.5	126.2	117.2	110.4	118.1
Fibre (5-6%)	61.6	62.4	48.0	55.6	53.0
NFE (nitrogen free extract) (calculated by difference)	378.7	351.0	384.9	389.0	374.6

\* Commercial *L. vannamei* feed (Starter I Pellets 933S Gold Coin; specifications: crude protein min., 36%; crude fibre max., 4%; crude fat min., 5%; ash max., 15%; moisture max., 12%); \*\* Values are mean of triplicate samples.

**Feeding trial.** A 1000 *L. vannamei* postlarvae (PL-10) were obtained from a commercial hatchery (PT. CPP Hatchery, Sluke, Rembang, Central Java Province, Indonesia) and were transported to the indoor nutrition laboratory facilities of the Center for Development of Brackish Water Aquaculture in Jepara. The PL-10s were acclimatized in laboratory conditions and maintained with a commercial diet (the diet A without the algae extract) for 45 d in two-500-L circular fiberglass tanks till they attain 2-3 g size. After acclimatization, the juveniles were graded by weight and groups of 10 shrimp with an average initial weight of 2.65±0.11 g were stocked in 15 black round plastic tank (30 L). The tanks were substrate-free flat-bottom plastic tanks equipped with continuous aeration and a black plastic mesh lid to minimize disturbances and prevent shrimp from jumping out. Five experimental diets were assigned to triplicate tanks in a completely randomized design.

Prior to start of the feeding trial, shrimp were acclimated to experimental diets and conditions for a 7-d period. Shrimp were fed three times daily *ad libitum* at 08:00 (30% portion), 13:00 (30% portion), and 17:00 (40% portion) on an initial feeding allowance of 8% total body weight d<sup>-1</sup> for 42-d. Shrimp that died during the experiment were replaced by tagged shrimp from the reserve tanks to avoid density dependent effects, but measurements were not made for the replacement shrimp (Sudaryono et al 1995, 1999a, 1999b). All shrimp in reserve tanks were fed with a commercial diet prior to being introduced into experimental tanks. The amount of feed given to each tank was determined for each group of ten shrimp.

The wet weights of individual shrimp were recorded at every 14-d interval to adjust the amount of feed given. Shrimp were allowed to feed for 3 h and then the uneaten food remaining on the bottom of the tanks was siphoned out, separated from other waste materials (faeces and exuvia), collected, redried, and weighed for determination of the total estimated feed intake. The bottom of all tanks was cleaned up by siphoning the faecal materials and residual feed debris before every feeding time.

The feeding trial was conducted at ambient temperature and a 12-h light:12-h dark photoperiod was maintained throughout the trial. Water quality parameters were monitored every-3 d for temperature, pH and salinity, everyweek for dissolved oxygen and every two weeks for total ammonia nitrogen. Water temperature ranged from 27.3-29.1°C, pH from 7.9-8.2 and salinity from 32.5-35.0 g L<sup>-1</sup>, dissolved oxygen 3.61-6.21 mg L<sup>-1</sup>, and total ammonia nitrogen < 0.01 mg L<sup>-1</sup>.

**Performance evaluation.** Diet performance was evaluated by calculation of weight gain (g/shrimp) = [final wet weight-initial wet weight (g)]; average daily growth (ADG;

mg/day per shrimp) = [weight gain (g)/growing period (day)]; estimated total feed intake (g/shrimp) = [(dry feed given – dry uneaten feed) (g) for feeding period]; feed conversion ratio (FCR) = [estimated total dry feed intake (g)/wet weight gain (g)]; survival (%) = [(number of shrimp surviving at the end of the study/number of shrimp at the beginning of the study) x 100]. Protein efficiency ratio (PER) was calculated by a formula PER = [wet weight gain (g)/dry protein intake (g)].

**Statistical analysis.** All data were analyzed by one way analysis of variance (ANOVA) and Duncan's multiple comparison test using a statistic program of SPSS version 21 for Windows. All probability values were set at 0.05 level of significance. Covariate ANOVA was used to demonstrate that there was no effect of initial shrimp weight on the observed parameters.

**Results and Discussion.** Growth performance, feed utilization, survival of shrimp after a 42-day feeding with diets supplemented with various levels of *S. cristaefolium* extract are summarised in Table 2. Average initial shrimp weights of each experimental group were similar ( $p > 0.05$ ) with a range of 2.56-2.76 g shrimp<sup>-1</sup> (2.65±0.11 g). No significant effect on final weigh gain (10.59-11.28 g shrimp<sup>-1</sup>) was shown by shrimp fed 5 different test diets. Dietary supplementation levels of 200, 600 and 1000 mg *S. cristaefolium* extract kg<sup>-1</sup> diet did not significantly affect ( $p > 0.05$ ) on weight gain and ADG performances of shrimp with a range of 7.84-8.36 g shrimp<sup>-1</sup> and 187-199 mg day<sup>-1</sup> shrimp<sup>-1</sup>, respectively. Similar growth performances were also shown by diets with adding 0 mg and 1400 mg extract kg<sup>-1</sup> diet and the 0 mg algae extract-based diet showed a higher growth performance that the diets with 200-1000 mg algae extract suuplementation. Similar performances in feed intake were also shown by the diets A (0 mg) and E (1400 mg).

Table 2

Growth response, feed intake, feed conversion ratio (FCR), protein efficiency ratio (PER), survival and moulting number of juvenile *L. vannamei* fed diets supplemented with various levels of tropical brown algae, *S. cristaefolium*, extract for 42 days

Paramater	<i>S. cristaefolium</i> extract supplementation levels (mg kg <sup>-1</sup> diet)				
	0 (Diet A)	200 (Diet B)	600 (Diet C)	1000 (Diet D)	1400 (Diet E)
Initial weight (g shrimp <sup>-1</sup> )	2.56±0.06 <sup>a</sup>	2.66±0.05 <sup>a</sup>	2.64±0.14 <sup>a</sup>	2.76±0.09 <sup>a</sup>	2.63±0.13 <sup>a</sup>
Final weight (g shrimp <sup>-1</sup> )	11.28±0.38 <sup>a</sup>	10.70±0.09 <sup>a</sup>	11.00±0.53 <sup>a</sup>	10.59±0.51 <sup>a</sup>	10.23±0.35 <sup>a</sup>
Wight gain (g shrimp <sup>-1</sup> )	8.72±0.35 <sup>a</sup>	8.04±0.25 <sup>bc</sup>	8.36±0.41 <sup>abc</sup>	7.84±0.45 <sup>c</sup>	8.60±0.23 <sup>ab</sup>
Average daily growth (mg day <sup>-1</sup> )	207.7±8.2 <sup>a</sup>	191.4±1.2 <sup>bc</sup>	194.0±9.9 <sup>abc</sup>	186.6±10.6 <sup>c</sup>	204.8±5.4 <sup>ab</sup>
Feed intake (g)	20.77±0.34 <sup>a</sup>	14.45±1.90 <sup>b</sup>	16.66±2.16 <sup>b</sup>	15.02±0.11 <sup>b</sup>	21.23±0.68 <sup>a</sup>
FCR	2.38±0.06 <sup>a</sup>	1.80±0.23 <sup>b</sup>	1.99±0.22 <sup>b</sup>	1.92±0.12 <sup>b</sup>	2.47±0.11 <sup>a</sup>
PER	1.17±0.03 <sup>b</sup>	1.56±0.22 <sup>a</sup>	1.38±0.15 <sup>a</sup>	1.45±0.09 <sup>a</sup>	1.13±0.05 <sup>b</sup>
SR (%)	80.0±10 <sup>a</sup>	86.7±5.8 <sup>a</sup>	96.7±5.8 <sup>a</sup>	96.7±5.8 <sup>a</sup>	90±10 <sup>a</sup>
Moulting (no. of shrimp)	42.7±2.08 <sup>a</sup>	39.3±4.07 <sup>a</sup>	36.3±3.21 <sup>a</sup>	48.0±14.7 <sup>a</sup>	44.3±6.03 <sup>a</sup>

Values are means + SD of three replicates. Mean values having the same superscript are not significantly different ( $p > 0.05$ ).

In contrast, shrimp fed with the diets supplemented with various levels of 200-1000 mg *S. cristaefolium* extract kg<sup>-1</sup> diet (diets B, C and D) have resulted significantly ( $p < 0.05$ ) in a better feed utilization (FCR 1.8-2.0; PER 1.40-1.56) than those fed with the diets A (0 mg) and E (1400 mg) with values of FCR (2.4-2.5) and PER (1.13-1.17). Survival and the number of moulting shrimp of all the treatment groups were high (80-97% and 36-48 shrimp, respectively) and not affected by *S. cristaefolium* extract supplementation levels. In addition, all test diets did not affect on survival and the number of moulting of shrimp

under a rearing period of 42 days with the values of 80-97% and 36-48 shrimp, respectively.

The efficacy of alginate and fucoidan as immunostimulant supplemented in diets for various shrimp has been well documented. It has been reported that dietary inclusion levels of 500-1000 mg alginate and fucoidan extracted from brown algae (*Sargassum* sp.)  $\text{kg}^{-1}$  diet were significantly effective to enhance immune response and the resistance of penaeid shrimp *P. monodon* and *M. japonicus* (at stadia postlarvae and juvenile) against bacterial (*Vibrio harveyi*, *Vibrio parahaemolyticus*, *Staphylococcus aureus*) and viral (WSSV) infections (Traifalgar et al 2009; 2010). Ghaednia et al (2011) also reported that shrimp *Fenneropenaeus indicus* immersed in hot-water extract of brown algae *Sargassum glaucescens* at 300 and 500  $\text{mg L}^{-1}$  for 2 h had improved the activity of haemocyte phagocytes and antibacteria. Similar results of the superiority of alginate and fucoidan as immunostimulant in penaeid shrimp have been also reported by Takahashi et al (1998) for *M. japonicus*, Chotigeat et al (2004) for *P. monodon*, and Cheng et al (2004) for *L. vannamei*. However, these previous studies were conducted in short durations and effects of alginate extracted in hot water from brown algae *S. cristaefolium* on growth and feed utilization have not been determined yet to date. So far according to the best of our knowledge, the present study is the first to document the effects of dietary *S. cristaefolium* hot-water extract supplementation on growth performance and enhancement of feed utilization efficiency in juvenile *L. vannamei*.

In the present study, survival, final weight and the moulting number of shrimp after the feeding trial was not affected by dietary *S. cristaefolium* extract supplementation but FCR and PER were significantly improved. Weight gain and ADG of shrimp fed the diets with no *S. cristaefolium* extract supplementation and the highest supplementation level of 1400  $\text{mg kg}^{-1}$  diet were higher than those fed the diets with 200-1000  $\text{mg kg}^{-1}$  supplementation. Enhanced weight gain and ADG in the present study are apparent at 0  $\text{mg kg}^{-1}$  and 1400  $\text{mg kg}^{-1}$  supplementation due to higher feed intake of shrimp fed with those diets. It was normal that the increased feed intake (more feed nutrition consumed) will increase growth of shrimp. However, in terms of feed utilization, these diets were poorer utilized by shrimp in comparison to shrimp fed the diets with 200 up to 1000  $\text{mg kg}^{-1}$  supplementation. This indicates that dietary 200-1000  $\text{mg kg}^{-1}$  supplementation of *S. cristaefolium* hot-water extract is adequate and effective to enhance the utilization of all nutrition available in the diet. These diets B, C and D are proven effective in improving FCR and PER, meaning that shrimp fed the diets supplemented with 200-1000  $\text{mg S. cristaefolium}$  extract  $\text{kg}^{-1}$  could utilized dietary nutrition including protein more efficient. This finding is in agreement with previous reports of Traifalgar et al (2009, 2010), indicating enhanced feed utilization (FCR and PER) promoting benefits of dietary *S. cristaefolium* extract application in shrimp. Similar feed utilization enhancement was also observed in *P. japonicus* fed peptidoglycan-supplemented diets (Itami et al 1989), in *P. monodon* larvae fed *Vibrio vulnificus* bacterin-supplemented diets (Sung et al 1994) and in juvenile *L. vannamei* fed Ergosan-supplemented diets, a commercial product prepared from a brown alga, *Laminaria digitata* (Montero-Rocha et al 2006). Enhancement of nutrient digestibility, resulting in efficient feed and protein utilization has been also reported by Crus-Suarez et al (2000) using juvenile *L. vannamei* fed diets supplemented with *Macrocystis pyrifera* as polysaccharide source. The latest study by Hafezieh et al (2014) also reported that no significant differences among treatments were found on weight gains in biomass ranged from 106.49 to 124.36  $\text{g L}^{-1}$  and SGR ranged from 4.68 to 5.68% as affected by supplementing *Sargassum illicifolium* meal for feeding juvenile shrimp *L. vannamei*. But shrimp fed with the test diets containing 10-15% *S. illicifolium* seaweed meal exhibited better feed conversion (1.15:1 and 1.17:1) than those containing 5% and no seaweed (control) (Hafezieh et al 2014). However, in fact, the mode by which these compounds promote efficient feed (FCR) and protein utilization (PER) in penaeid shrimp is not yet fully explained and understood.

Feed utilization enhancement effects of dietary *S. cristaefolium* polysaccharide extract might be attributed to the efficient nutrition and assimilation caused by the activation of fixed phagocytes in the hepatopancreas that are known to produce lytic

enzymes upon stimulation (Azad et al 2005). Health improvement as affected by immunostimulant supplementation might have been attributed to reduced stress from opportunistic microbial pathogens so this will result in good survival and growth of immunostimulated aquatic animals (Sung et al 1991; Itami et al 1989).

**Conclusions.** Nevertheless, the present findings showed that dietary *S. cristaefolium* extract supplementation (not more than 1000 mg kg<sup>-1</sup>) could enhance feed utilization efficiency (FCR; PER) of juvenile *L. vannamei*. Based on results of this present work, dietary *S. cristaefolium* extract supplementations of 200-1000 mg kg<sup>-1</sup> are recommended to reduce feed cost in juvenile *L. vannamei* culture due to the use of aquaculture feed can be saved up to 22%. However, the mechanisms involved on how this compound improves FCR and PER in shrimp and the digestibility study is yet to be clarified for further investigations.

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