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Potential of using tropical brown macroalgae *sargassum cristaefolium* meal in the diets for juvenile white shrimp (*litopenaeus vannamei*)

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Abstract. The objectives of this study were to evaluate the effect of different dietary levels of tropical marine brown macroalgae, *Sargassum cristaefolium* meal (SCM) on growth performance and feed utilization efficiency of juvenile white shrimp, *Litopenaeus vannamei* (mean initial weight 2.65±0.1 g). The algae were collected from Bandengan Coast, Jepara, Indonesia. The study used a completely randomized design with 5 treatments of dietary different *S. cristaefolium* meal levels (0, 10, 20, 30, and 40 g algae meal/kg feed) in triplicates. The results showed that the different levels of dietary SCM did not significantly affect (P>0.05) average daily growth (ADG;.204-224 mg/day) and survival (80-97%) of the shrimp after a 42-day feeding period. However, the test diets significantly affected (P<0.05) feed intake (FI), feed conversion ratio (FCR), and protein efficiency ratio (PER). Better FCR (2.13) and PER (1.34) was found by the diet with 2% SCM compared to the control diet (no SCM) (FCR 3.42 and PER 0.81). This finding indicates that the supplement of dietary 2% SCM (20 g/ kg feed) can increase the feed utilization efficiency up to 62% or reduce the FCR up to 38% without any adverse in growth performance.

1. Introduction

The white shrimp, *Litopenaeus vannamei* is currently to become the major species being cultured worldwide [1, 2]. Bacterial and viral diseases outbreaks are a major problem faced by penaeid shrimp aquaculture industry especially penaeid shrimp, *L. vannamei*. In recent decade, the industry has got many serious economic losses due to the microbiological diseases causing high mortality in all producing countries [3, 4, 5). Reducing environment quality of commercial intensive shrimp aquaculture may have resulted in increased microbiological diseases.

Reducing the use of antibiotics due to the problems of antibiotic residual in food and resistant pathogens spreading in aquaculture environment [3, 6] has resulted in more attention for the use of immuno stimulants [7, 8]. The use of immuno stimulating substances such as carrageenan, laminaran, alginate and fucoidan derived from marine seaweeds origin extract that could enhance immune response and promote growth in some penaeid shrimp species has been studied by many workers [1-4, 9-13]. However, only a few information regarding the evaluation of marine macro algal

polysaccharides on shrimp immune response, disease resistance and growth performance has been available so far. So that this work is needed to conduct.

The present study was conducted to evaluate the potential of using tropical brown marine macroalgae *Sargassum cristaefolium* meal in diets for growth performance and feed efficiency of white shrimp *Litopenaeus vannamei* juveniles.

2. Methods

This study was carried out from March to May 2017 at Coastal Eco-Development Laboratory, Faculty of Fisheries and Marine Science, Diponegoro University, Semarang, Central Java Province.

2.1. Preparation of marine brown algae meal

Marine brown algae *Sargassum cristaef*olium were collected from Bandengan Coast, Jepara, Central Java Province, Indonesia. After collection, the algae were washed thoroughly by freshwater to remove the debris and then dried under room temperature and powdered using an electric grinder. The meal was packed and stored until use.

2.2. Test shrimp and diets

White shrimp *L. vannamei* postlarvae (PL-12) were obtained from a commercial hatchery (PT. CPP Hatchery, Sluke, Rembang, Central Java Province, Indonesia) and transferred to the indoor Nutrition Laboratory, the Center for Development of Brackish Water Aquaculture, Ministry for Marine and Fisheries Affairs, Jepara. To make sure that the shrimp were in good health condition, the shrimp were tested PCR negative (nested PCR). The shrimp were reared in six tons capacity concrete tank as stock tank containing 3000L of aerated seawater at salinity 32.5-35.0 ppt, with pH 8.0-8.2 and temperature 27.5-30.0°C for a period of 45 days until they reached juveniles size approximately 1.5-3.0 g before starting the feeding trial. During the nursery, the shrimp were fed a commercial *L. vannamei* feed (Starter I pellets 933S Gold Coin) containing crude protein minimum 36%, crude fibre maximum 4%, crude lipid minimum 5%, ash maximum 15% and moisture maximum 12%. A 20% daily water exchange was maintained during the nursery.

The commercial *L. vannamei* feeds as used in the nursery were used as basal diets in this study with the composition of test diets as shown in Table 1. Wheat flour was used to adjust to 100%% total proportion in the test diets.

Five experimental dry pellet diets containing similar approximately 36% crude protein (dry weight basis) were formulated to contain 0 (as a control), 10, 20, 30, and 40 g *S. cristaefolium meal* /kg diet. The procedure to prepare the test dry pellet diets followed the method conducted by [2]. The dried test diets were packed in plastic bags and stored in the refrigerator until used for feeding trial.

Table 1. Composition (g/k g) of experimental diets for *L. vannamei* juvenile

Ingredient	Composition (g/kg)				
	SD0	SD1	SD2	SD3	SD4
S. cristaefolium meal (0-4%)	0	10	20	30	40
CMC (binder) (0.5%)	5	5	5	5	5
Wheat flour	40	30	20	10	0
Basal diet* (95.5%)	955	955	955	955	955
Total (100%)	1000	1000	1000	1000	1000
Proximate composition (% dry weight basis)**					
Crude protein	35.6	36.9	36.2	36.5	35.8
Crude lipid	8.1	8.8	9.0	7.9	8.6
Crude ash	11.8	12.6	11.7	11.4	11.1
Fibre	6.5	6.2	5.8	5.7	5.9
NFE (calculated by difference)	38.0	35.5	37.1	38.5	38.6

* Commercial *L. vannamei* feed (Starter I Pellets 933S Gold Coin)
** Values are mean of triplicate samples.

2.3. Feeding trial and evaluation parameters

A total of acclimated one hundred and fifty shrimp juveniles with an initial individual mean wet weight of 2.65±0.07 g were randomly selected from the stock tank and transferred to 25-L capacity black round plastic experimental tanks filled with 20-L seawater for the feeding trial. Shrimp were randomly stocked at 10 individuals per tank in triplicates per treatment in 15 experimental tanks. The experimental tanks were substrate-free flat-bottom plastic tanks equipped with aeration and a black plastic net as a cover to minimize disturbances and avoid the shrimp from jumping out. During a period of feeding trial for 42 days, water quality in the experimental tanks were monitored with salinity ranged from 32-35 ppt, water temperature from 27.5-30^{o}C, dissolved oxygen from 3.70-6.50 mg/L, and total ammonia <0.01 mg/L. A 12 h light: 12 h dark photoperiod was maintained throughout the trial.

The feeding protocol of this work followed the method conducted by Sudaryono et al. [2]. The test diets were given to the juveniles three times a day *ad libitum* at 8:00 (30%), 13:00 (30%) and at 17:00 (40%) on a 8% initial feeding rate of total body weight for a 42 days period. Fecal wastes and uneaten diets were collected from the tanks using siphon every before the feeding time. Uneaten diets were dried and calculated to determine feed intake and feed efficiency ratio. Every 14 days interval, the shrimp were weighed to adjust the amount of feed given and counted to monitor the survival in each experimental tank. The molting shrimp were monitored daily to determine any effect of the treatment diets on molting frequency of the shrimp. At the end of the feeding trial, growth performance measured as weight gain (WG), average daily growth (ADG), protein retention (PER), feed conversion ratio (FCR), feed intake and survival were calculated as previously described by Sudaryono et al. [2].

2.4. Biochemical and statistical analyses

Crude protein of test shrimp carcass after feeding treatments and test diets were determined using by the Kjeldahl method (Tecator Kjeltec System 1007, Denmark). Crude lipid was determined by Soxhlet extraction method, ash and moisture contents were determined according to [14].

All data obtained from this study was statistically analyzed by one-way analysis of variance (ANOVA) and Duncan's multiple comparison test using a program of SPSS version 21 for windows. Probability values of 0.05 were considered significant. Covariate ANOVA was used to make sure that there was no effects of initial shrimp weight on the calculated parameters.

3. Results And Discussion

Growth performance, feed intake , FCR, PER, survival and molting frequency of shrimp as effect of feeding treatment with diets containing different levels of brown algae *S. cristaefolium* meal for 42 days are summarized in Table 2. Initial shrimp wet weight of test shrimp were similar (P>0.05) ranging from 2.62-2.69 g/juvenile with an average of 2.65±0.07g. There were no effects (P>0.05) of the diet treatments on final shrimp weight (11.2-12.0 g), weight gain (8.6-9.4 g), ADG (204-224 mg/day), survival (80.0-96.7%), and molting frequency (36.0-44.3%) after the feeding trial for 42 days. However, feed intake (FI), FCR, and PER were significantly affected (P<0.05) by different inclusion levels of dietary *S. cristaefolium* meal and shrimp fed with 2% *S. cristaefolium* meal based diet (SD2) resulted in the best feed efficiency performance. In addition, all shrimp fed with the control diet (SD0) gave worse responses (P<0.05) in terms of FI, FCR, and PER than those with the diets containing the brown algae meal more than 2%. Similar nutrient utilization performances (P>0.05) (FCR and PER) were also shown by shrimp fed 3% and 4% *S. cristaefolium* meal containing diets.

Dietary *S. cristaefolium* supplementation levels had no significant effects on shrimp carcass composition. There were no significant differences in shrimp carcass protein, lipid, and ash contents after shrimp were fed with different test diets for 42 days (Table 3).

Disease outbreak up to now is still to be a major problem in shrimp production. The efforts to overcome and prevent shrimp disease are still difficult and the methods to support these efforts are still poorly understood. Lack of the specific immune system in crustaceans has caused vaccines for crustaceans are practically not available. Therefore, more attention is focused on the benefit of using immuno stimulants as one of problem solvers in improving immune response in crustaceans [15]. Use of various immuno stimulants such as glucan, peptidoglycans, fucoidan, alginate, lippolysaccharides,

live bacteria, killed-bacteria supplemented in diets has been reported effective in strengthening the non-specific defense mechanism and protecting shrimp against disease [1, 2, 3, 4, 9, 10, 11, 12, 13, 15]. In addition, polysaccharides containing active compounds derived from marine brown algae (*Sargassum* spp.) extract and meal forms have been widely studied and documented having the ability to enhance disease resistance and promote growth in penaeid shrimp *Penaeus monodon* [3, 4] and *L. vannamei* [2, 11, 16].

Table 2. Growth performance, nutrient utililization efficiency, survival and molting number of *L. vannamei* juveniles after feeding with experimental diets supplemented with graded levels of *Sargassum cristaefolium* meal for 42 days

Paramater	Dietary *S. cristaefolium* meal supplementation levels (g/kg diet)				
	0 (SD0)	10 (SD1)	10 (SD1)	10 (SD1)	10 (SD1)
Initial weight (g/shrimp)	2.68 ± 0.02^{a}	2.62 ± 0.05^{a}	2.69 ± 0.04^{a}	2.63 ± 0.09^{a}	2.62 ± 0.12^{a}
Final weight (g/shrimp)	11.6 ± 0.4^{a}	11.2 ± 0.2^{a}	11.5 ± 0.5^{a}	12.0 ± 0.5^{a}	11.9 ± 0.5^{a}
Wight gain (g/shrimp)	8.9 ± 0.7^{a}	8.6 ± 0.5^{a}	8.8 ± 0.5^{a}	9.4 ± 0.5^{a}	9.3 ± 0.4^{a}
ADG (mg/day)	212 ± 17^{a}	204 ± 3^{a}	211 ± 13^{a}	224 ± 11^{a}	220 ± 10^{a}
Feed intake (g)	30.4 ± 1.9^{a}	28.6 ± 1.0^{ab}	18.3 ± 1.2^{d}	25.2 ± 1.6^{c}	25.8 ± 2.4^{bc}
FCR	3.4 ± 0.3^{a}	3.4 ± 0.1^{a}	2.1 ± 0.1^{c}	2.7 ± 0.1^{b}	2.8 ± 0.4^{b}
PER	0.81 ± 0.02^{c}	0.85 ± 0.04^{c}	1.34 ± 0.07^{a}	1.07 ± 0.04^{b}	1.03 ± 0.13^{b}
Survival (%)	96.7 ± 5.8^{a}	83.3 ± 5.8^{a}	93.3 ± 5.8^{a}	80.0 ± 10.0^{a}	90.0 ± 10.0^{a}
Moulting (no. of shrimp)	37.0 ± 7.0^{a}	39.0 ± 2.0^{a}	41.0 ± 4.0^{a}	36.0 ± 4.0^{a}	44.0 ± 2.0^{a}

Values are the means of triplicate group. Mean values having the same superscripts are not significantly different (P>0.05).

Table 3. Proximate composition of *L. vannamei* juveniles carcass fed with test diets supplemented with graded levels of *S. cristaefolium* meal for 42 days

Inclusion levels of brown algae meal	Crude protein (%)	Crude lipid (%)	Crude ash (%)
Carcass			
0	68.23 ± 0.20^{a}	6.63 ± 0.12^{a}	18.96 ± 0.07^{a}
10	68.10 ± 0.15^{a}	6.80 ± 0.16^{a}	20.23 ± 0.52^{a}
20	69.31 ± 0.59^{a}	6.73 ± 0.09^{a}	18.35 ± 0.45^{a}
30	68.18 ± 0.47^{a}	6.82 ± 0.04^{a}	19.06 ± 0.13^{a}
40	68.44 ± 0.37^{a}	6.75 ± 0.13^{a}	18.75 ± 0.64^{a}

Values are the means of triplicate group ± SD and calculated as % of the dry matter

Previous diet studies showed that dietary brown marine algae *Sargassum wightii* supplementations were found effective in supporting growth and survival of *P. monodon* [4]. Similarly, [3] also found that growth performance (weight gain, specific growth rate, FCR and PER) and survival of *P. monodon* postlarvae enhanced when the shrimp fed the 500-2000 mg fucoidan derived from brown algae *Undaria pinnatifida*/kg diet. In the present study, in contrast with the previous studies, supplementation of brown algae *S. cristaefolium* meal in the diet did not enhance growth (final weight, ADG, weight gain, total molting) and survival of *L. vannamei*. This present result is in agreement with the earlier work of [11] who reported that survival and weight gain of *L. vannamei* juveniles were not affected by addition of dietary sodium alginate at different levels (0, 0.5, 1.0 and 2.0 g/kg diet). Similar results were also shown by [16] that no significant differences in survival and weight gain of *L. vannamei* were observed when they consumed diets containing different levels (0, 5, 10, 15%) of

Sargassum illicifolium. Addition of *S. cristaefolium* extract at a dosis of 0, 200, 600, 1000, 1400 mg/kg diet did not significantly affect on the final weight and survival of *L. vannamei* during a 42-day feeding trial period [2]. This indicates that differences in growth performance and survival are influenced by differences in shrimp species, brown algae species and the type of immunostimulants.

However, quite interestingly that addition of *S. cristaefolium* meal in the diet improved FCR and PER of *L. vannamei* in the present study. A dose of 20 g *S. cristaefolium* meal/kg diet in the present study resulted in the best FCR (2.13) and PER (1.34) compared to doses of 0-10 g and 30-40 g *S. cristaefolium* meal/kg. This indicates that 20 g algae meal/kg supplementation is adequate in promoting nutrient utilization efficiency in this study. At that concentration, shrimp showed an optimum nutrient utilization level so that it resulted in reducing FCR and increasing PER. In fact, addition of 20 g algae meal/kg diet significantly promotes better nutrient utilization efficiency. The lowest feed intake is apparent at 20 g/kg supplementation with no differences in growth as affected by increasing the levels of dietary algae meal. Lower feed intake to produce similar growth in the present study is a proof that enhancement of nutrient digestibility might have occurred, resulting in more efficient nutrient utilization including protein. Similar performance was also shown by earlier work of [16], better FCR (1.15-1.17) were obtained in *P. monodon* fed diets containing dietary increased algae levels for 45 days. Feed utilization efficiency enhancement (lower FCR) was also observed in *L. vannamei* juveniles fed *S. cristafolium* extract-supplemented diets [2]. Despite these reports, the mode by which these bioactive compounds promote feed utilization efficiency (FCR) and protein utilization rate (PER) in white shrimp *L. vannamei* is not yet fully explained.

The process of efficient nutrient utilization through digestion and assimilation caused by the activation of fixed phagocytes in the hepatopancreas to produce lytic enzymes upon stimulation might have been attributed to feed utilization enhancement effects of dietary *S. cristaefolium* meal [10]. Enhancement of health status caused by increased active immunological defense and stress tolerance from opportunistic microbial pathogens might have been attributed to the bioavailability of active compounds (immunostimulants) contained by polysaccharides derived from brown marine macroalgae *Sargassum* spp. consumed by the cultivated species [3, 11, 16]. Moreover, the present findings show that dietary *S. cristaefolium* supplementation can promote nutrient utilization efficiency of white shrimp *L. vannamei* juveniles. With limitation and restriction in using antibiotics in aquaculture, utilization of tropical brown marine macroalgae *S. cristaefolium* meal as a feed supplement can be one of problem solvers to overcome disease problem in shrimp production. However, how the active compounds of polysaccharides derived from tropical brown marine algae *S. cristaefolium* meal can enhance feed utilization efficiency in *L. vannamei* juveniles have not been understood yet and this should be addressed for further investigations.

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