The effect of brown seaweed (*Sargassum ilicifolium*) powder on western white leg shrimp

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Received: March 2015 Accepted: September 2016

Abstract

The effects of substitution of seaweed, *Sargassum ilicifolium*, by replacing protein resources, in *Litopenaeus vannameii* diets was studied. It was carried out by incorporation of raw powdered seaweed at three levels, 0% as control treatment, 5% (C), 10% (B) and 15%(A) each with four replications in isoprotein, (33%) and isocaloric (355kcal.100^{-1}) diets.. Binder properties of seaweeds in different diet pellets were evaluated. In laboratory conditions, 480 shrimp juveniles (initial weight=3 gram) acclimated in 16 plastic tanks, fed 4% of their biomass daily. During the 45 day digestibility experiment, biometric indices were measured at 15 day intervals to assess the growth performances. Based on physicochemical analysis of water at 2 day intervals, effective parameters were maintained at a required range for the wellbeing of the shrimp during the experiment. Results of the experiment show that *Sargassum* sp. can be used as a binder in shrimp feed ingredients with significant differences between treatment pellet stability and water absorbance percent in sea water.Although initial biomass, SGR and survival rate showed no differences between treatments, but final biomass , biomass gain, FCR, shrimp flesh color and cholesterol levels revealed significant differences between treatments (p<0.05). The diet with the highest level of seaweed (A) showed not only the best growth performances, survival rate, change in the flesh color desirable to the consumers and decreased cholesterol level in shrimp, but also has the best binder property for shrimp pellet making.

Keywords: *Sargassum ilicifolium*, Replacement, *Litopenaeus vannameii*, Protein resource, Oman Sea

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Introduction

Aquaculture production in I.R of Iran increased expeditiously from 3219 tons in 1978 to 371840 tons in 2014 with approximately 40% of total fish production. It is expected to increase to 46% during the sixth five-year developing program starting from 2017 (FAO, 2016). Of the total aquaculture production, production of western white leg shrimp, *Litopenaeus vannameii*, the only cultured shrimp in Iran, represented 6 percent, 22475 tons in 2014 (FAO, 2015). Production of shrimp in the previous year was 48% lower than that in 2014 and the high prices of shrimp in the world market have been one of the major factors for this fantastic increase in production.

On the other hand there are more than 180 different species of brown, red and green seaweeds in the coastline of the Persian Gulf and Oman Sea of which *Sargassum* spp. classified in the brown seaweed group has the highest resources especially in Sistan and Baluchistan coastline, intensive in Chabahar to Konarak Bays (Gharanjic and Rohani, 2010). Based on seaweed stock assessment done by Off-Shore Fisheries research center- Chabahar, the total biomass of *Sargassum* sp. In 2005 and 2014 were 2000 and 500 tons respectively. Since seaweeds have proteins, vitamins, carbohydrates, fiber, lipid and mineral richness, and are used to feed different species and in form of wet and dry (Diaz-Peferrer and Lopez, 1961; Halperin, 1971; FAO, 2005; Hafezieh *et al*., 2014), there have been many research works using seaweed in fish and shrimp diets. Cruz-Suarez *et al*. (2000) used large brown algae, *Macrocystis pyrifera* powder in shrimp *L. vannameii* feed ingredient. Also He and Lawrence (1993) added the flavor of *Laminaria digitata* in feed ingredient of this shrimp. Tahil and Juinio-Menez (1999) used different seaweeds as feed for gastropoda.

Materials and methods

All the experiments were done in the Off-Shore Fisheries Research Center-Chabahar (OSFRC). Seaweed *Sargassum ilicifolium* was collected from Tis Beach, Chabahar Bay- Oman Sea, Sistan and Baluchistan coastline, washed and dried at 5°C for 36h, then powdered by grinder to 200 micron size and used as proportional feed for 500 juvenile shrimp (*Litopenaeus vannameii*) which were acquired from a shrimp farm of Jusk port, Hormozgan province and transported using standard methods, in plastic bags with 2/3 oxygen and 1/3 water to the laboratory of the OSFRC. Shrimp were then kept in a 5000 L tank with constant aeration and fed at 4% biomass for seven days acclimatization. After 7 days, they were divided into 16 tanks (30 juveniles in each 300 L tank) equipped with aeration using two 3L min^{-1} air pumps in order to maintain stable physic-chemical conditions and fed ad libitum with a commercial Havorash shrimp feed as control and three experimental feeds, for the 45 day digestibility experiment. The experiment was set up in a randomized design and water was treated with activated carbon filters.

Tanks were siphoned daily to remove bottom residue and organic wastes. Tanks were illuminated with fluorescent light, maintaining a 14:10 h L: D photoperiod. Physical and chemical parameters such as DO, WT, Salinity and pH were measured at 2 day intervals.

Formulation and preparing of diets

The diets were formulated as follows: algae flour (made from the *Sargassum* algae), soybean meal and oil, corn flour, fish flour, meat and bone meal, wheat flour, cassava flour, mineral and vitamin blend, and iodized salt. The four treatments contained different proportions of seaweed flour and soy, fish and wheat flours (Table 1). Formulation of the isoprotein (33%) and isoenergy (355 kcal.100^{-1}) diets was finalized following procedures described by Correia (2004), EMBRAPA (1989) and the National Research Council (1989), that were also employed in the seaweed analyses (Tables 2 and 3). The ingredients were ground to 200 micron size powder, homogenized with 40% water at 60°C, placed in a meat mincer pellet former of 2 mm diameter and dried in an oven at 60°C for 24h. FCR was determined by the amount of feed ingested divided by the weight gain of shrimp; survival rate calculated from the number of live shrimp at the end of experiment, and SGR was measured by the formula:

$SGR=100\ (\ln P_f - \ln P_i)/\ t$

Where P_f is the final weight, P_i is the initial weight, and t is time.

Table 1: Proportion of ingredients of the experimental diets used to feed the shrimp *Litopenaeus vannamei.*

Ingredients (%)	Diets			
	A	B	C	D
Seaweed flour	15.0	10.0	5.0	0.0
Soy flour[a]	7.0	12.0	16.0	22.0
Fish flour[a]	37	33	30	26
Wheat flour	13.3	19.0	25.0	27.0
Meat and bone flour[a]	8.0	8.0	8.0	8.0
Corn flour[a]	8.0	8.0	8.0	8.0
Cassava flour[a]	7.0	7.0	7.0	7.0
Soy oil	1.0	1.0	1.0	1.0
Vitamin and mineral blend[b]	1.0	1.0	1.0	1.0
Iodated salt	0.5	0.5	0.5	0.5

[a] Percentage composition according to supplier: soy flour – CP 44.84;DM 88.22; EE 1.74; F 5.57; A 5.73; DE 3,005 kcal kg^{-1} ; fish flour –CP 54.06; DM 92.89, EE 15.30, F 1.51, A 22.92, DE 33,335 kcalkg^{-1} ; wheat flour – CP 16.76, DM 87.74, EE 3.13, F 8.12, A 4.57,DE 2,930 kcal kg^{-1} ; meat and bone flour – CP 40.60, DM 91.00, EE 16.00, F1.51, A 36.60, DE 2,929 kcal kg^{-1} ; corn flour – CP 8.68,DM 87.45, EE 3.84, F 2.17, A 1.18, DE 3,110 kcal kg^{-1} ; cassavaflour – CP 5.84, DM 5.84, EE 0.55, F 13.83, A 1.55, DE 2,771 kcal kg^{-1} (CP crude protein; DM dry matter; EE ether extract, F fiber, AAsh, DE digestive energy)b Guaranteed levels per kilogram of product: vitamin A 900,000 IUkg^{-1} , biotin 6.0 mg, vitamin B1 150 mg, vitamin B2 600 mg, vitaminB6 300 mg, vitamin B12 1,200 mg, E 2,000 IU kg^{-1} , niacin 2,500 mg,folic acid 80 mg, pantothenic acid 1,200 mg, selenium 25 mg.

Table 2: Proximate analysis of Sargassum. CP Crude protein, EE ether extract, M moisture; C carbohydrate, Min minerals (g.100 g^{-1} DW of seaweed) and caloric value (Kcal.100 g^{-1}).

Ingredients (%)	*Sargassum* sp.
Crude protein (N*6.25)	9.18±1.15
Crude fat	2.11±0.43
Total fiber (a)	10.34±2.21
Ash	29.15±3.43
Carbohydrate	33.11±2.03
Humidity	16.11±1.00
Caloric value	235.1 ±7.12

(a): calculated by100-(Crude Protein+Crude Fat +Ash+Carbohydrate)

Table 3: Calculated composition of some nutrients and digestive energy in the experimental diets.

Nutrient diet	A	B	C	D
Digestive energy (kcal 100 g^{-1})	355.5	356.0	356.2	356.5
Crude protein (%)[a]	33.12	33.12	32.98	33.05
Ether extract (%)[a]	7.36	6.78	6.21	5.64
Raw fiber (%)[a]	9.42	8.17	6.92	5.66
Ash (%)[a]	11.12	10.35	9.59	8.82

[a] Percentage of nutrient in diet

The results regarding gains in biomass, specific growth, survival and feed conversion were assessed by analysis of variance (ANOVA) complemented with the Tukey test (α=0.05).

Chenmical analysis of seaweed, diet and shrimp

Water stability and absorption capacity of the pellets in sea water were measured by placing 2 g pellet of experimental feed, each with three replications in plastic glasses with seawater, recording the time and percent of pellet leaching and water absorption percentages after one hour (AOAC, 1990).

Astaxanthine measurement (ppm) in diets was done using standard spectrophotometry (Schuep and Schierle 1995). Total nitrogen, fiber, and ash contents were determined by standard AOAC (1990) methods. Fat content was determined according to Bligh and Dyer method (Bligh and Dyer, 1959). Total protein content was calculated by multiplying Kjeldahl nitrogen by 6.25. Ash content was conducted by blasting the ground dried samples overnight in a muffle furnace at 525±0°C. Crude fiber analysis was determined by filtering with a Fiber-Tec system. Shrimp muscle colorimeter was done by HPLC (Torrison & Naevdal, 1984). Six individual shrimp were collected randomly from each treatment and 200-300 grams of its muscle was removed, put in acetone with 1.5% sodium sulphate, maintained at 4°C for 3 days, centrifuged at 5000 rmp for 5 minutes and finally colorimetric with spectrophotometer. Spectrum was valued from 1 for white, 2 for pink to 3 for orange color. Cholesterol level of shrimp flesh was

analyzed using standard methods (AOAC, 1990).

Results

The shrimp exhibited satisfactory growth under all conditions tested. Growth was assessed through the data on final biomass, gains in biomass and specific growth rate (SGR), under the four tested conditions.

Other analytical measurements including stability, seawater absorption and astaxantine of pellet, cholesterol and colorimetric of shrimp flesh were exhibited in Table 4.

Table 4: Stability and seawater absorption of the pellets (%), growth, survival, colorimetric, astaxanthine (ppm) and cholesterol content data on shrimp fed with different feeds (mean±standard deviation, n=100 per treatment group). Different letters on the same line indicate statistical differences ($p \leq 0.05$).

Data Treatment	A	B	C	D
Stability in seawater%	98[a]	97[a]	95[b]	95[b]
Seawater absorption%	110[a]	100[b]	85[c]	80[c]
Initial biomass (g .tank^{-1})	90.10±0.08 [a]	90.42±0.15 [a]	91.78±0.15 [a]	90.77±2.12 [a]
Final biomass (g. tank^{-1})	350.27±2.97 [a]	355.82±2.03 [a]	360.89±2.08 [a]	360.66±3.19 [a]
SGR (%/day)	3.08±0.85 [a]	3.04±0.71 [a]	3.09±0.96 [a]	3.05±0.45 [a]
Gain in biomass (g. tank^{-1})	259.50±3.15 [a]	268.22±2.72 [a]	265.40±1.97 [a]	270.56±3.11 [a]
Survival (%)	95.20±6.73 [a]	97.00±2.73 [a]	97.00±2.73 [a]	96.20±4.18 [a]
Feed conversion ratio[c]	1.15±0.0 [a]	1.17±0.03 [a]	1.33±0.17[b]	1.30±0.22 [b]
Colorimetric[d]	c[a]	c[a]	b[b]	a[c]
Cholesterol (mg.100 $^{-1}$g ww)	121.68±12.121[a]	127.54±14.33[b]	130.84±10.25[c]	147.92±11.01[d]
Astaxanthine (ppm)	104.10±1.00[a]	103.11±1.00[a]	90.19±1.00[b]	0.0±0.00[c]
Cultivation periods (days)	45	45	45	45
Juvenile density /300 L. tank	30	30	30	30

[c] Total feed supplied in dry weight /biomass gain in wet weight.
[d] Spectrom from 1 for white, 2 for pink and 3 for orange color.

Discussion

The present study assessed the replacement of fish protein resources, soy and wheat meals by seaweed in diets of juveniles of *L. vannameii*. The physical-chemical variables remained within the range recommended for *L. vannamei* by Clifford (1992), Rocha *et al.(*1998), Barbieri *et al.* (2001), Álvarez *et al*.,(2004) and McGraw and Scarpa (2004), such that these variables did not interfere with the treatments.

In previous research, the authors showed that the substitution of artificial feed binders with seaweed meal increased the water absorption of shrimp pellets and improved their texture while maintaining adequate pellet integrity (Briggs and Funge-Smith, 1996; Penaflorida and Golez, 1996; Cruz-Suarez *et al.*, 2000; Wong and Chaung, 2001a, b; Cerecer-Cota *et al.*, 2005; Suarez-Garcia, 2006; Marinho-Soriano *et al.*, 2007).

Growth rates of the experimental shrimp observed during the present trials ranging from 0.15 to 0.2 g.day^{-1} were the same as previously published data, which ranged from 0.11 to 0.19 g/day (Smith *et al.,* 1985) to 0.26 g.day^{-1} (Menz and Blake, 1980). Pedreschi-Neto (1999) also evaluated 0.11 and 0.20 g for final biomass and 1.68 to 3.17% SGR using shrimp larvae in a 60 day experimental period. The decreasing growth rates over the trial period compared to some studies may be attributed to approaching adulthood and partly to a slight reduction of daily food administration over the last two weeks of the growth trial. Other results from Pedreschi- Neto (1999) showed a survival rate ranging between 51.7% and 60% (average 55.35%) which were below the survival percentages of the present study.

Cornejo *et al.* (1999) evaluated the effect of the seaweed *Caulerpa sertularioides* on *Penaeus californiensis* and concluded that the presence of the algae has a direct effect on growth, survival and biomass of the brown shrimp *P. californiensis* under laboratory conditions.

Cruz-Suarez *et al.* (2000) observed 100% survival rate in shrimp treatments fed with feed supplemented with seaweed. Feed conversion using *Phaeophycea* algae flour was 2.63±0.42; 2.80±0.27 and 3.12±0.54, using 0, 4% and 8%, algae in shrimp feed respectively. These values were lower than those of the present experiment for survival.

The increasing concentration of raw *S. ilicifolium* resulted in reduction in mean weight gain and feed efficiency, change in the shrimp flesh coloration and decreased cholesterol of shrimp. Previously (Marinho-Soriano *et al*., 2007) reported that *G. cervicornis* could be effectively used as a partial substitute for industrial feeds in shrimp *L. vannamei*. The Indian white shrimp fed with seaweed incorporated diet (*Ulva lactuca* and *Sargassum wightii*) showed improved survival and resulted in higher SGR (Mukhopadhyay and Ray, 1999). The higher levels of incorporation (20 % and 30 % seaweed) did not perform well. The reduced growth of the prawn fed the diets containing higher levels of raw seaweeds appeared to be due to increasing fiber content of seaweeds in the diets. Seaweeds are the cheapest protein sources but their utilization is limited by the presence of high amount of crude fiber which can be eliminated by fermentation process (Felix and Brindo, 2008). The best performance of shrimp in terms of SGR, and FCR, was observed in the diets containing higher levels of seaweed but differences with other treatments were not significant.

Cholesterol and lipid carcass composition of shrimp can be reduced with the consumption of *Sargassum* meal (Casas-Valdez *et al*., 2006) and *Ulva clathrata* (Cruz-Suarez, 2008). Ulva is known to change fat deposition and metabolism patterns in sea bream (Nakagawa *et al*., 1987) apparently resulting in a more efficient use of fat

deposits so weight loss during winter stress is reduced, as well as other compositional changes. The effect may be due at least in part to cysteinolic acid, a non-protein amino acid similar to taurine. Cysteinolic acid, like taurine, can form conjugates with cholesterol in the formation of bile dalts, at least in sea bream (Une *et al*., 1991)

Color of shrimp flesh was changed from white to orange in control to heist 155 seaweed inclusion of feed, respectively. The astaxanthin content in muscle tissue was higher in shrimp fed with 15 and 10% *S. ilicifolium* replaced by protein resources of shrimp diet. This seaweed contains 1207.78 ppm astaxanthin (Hafezieh *et al*., 2014). Carotenoids, particularly astaxanthin content of feeds are one of the major factors influencing the color development in animals (Moretti *et al*., 2006). But at the same time scientific knowledge about several factors like dietary pigment source, their dosage level, feeding duration, dietary composition and magnitude of carotenoid esterification is also required to identify these interaction processes (White *et al*., 2002).

In conclusion, it is evident from the present study that seaweeds can be utilized as feed ingredients in the diets of juveniles of *L. vannameii* without any compromise in growth performance and feed utilization efficiency.

Acknowledgments

Many thanks to Vice-President of Science and Technology and Iranian Fisheries Research Science Institute for financially supporting this study and for providing laboratories and equipment for this project. Our thanks are due to Havoorash shrimp feed factory for supplying the ingredients and making the diets and all our colleagues at the Off-Shore Fisheries Research center for their cooperation.

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