Using Seaweed *Sargassum illicifolium* in Shrimp *Litopenaeus vannameii* Aquaculture

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Abstract. The marine brown seaweed *Sargassum illicifolium* is abundant along the Iranian coastline of Oman Sea which is rich in nutrients. The aim of the present study was to test the hypothesis which it canbe used as a source of protein in shrimp diets. The experiment was conducted in a laboratory, where 3 grams shrimp juvenile acclimation in a 5,000 L tank. They were then kept in plastic tank each containing 300 L water and 30 shrimp juveniles were fed daily (4% of biomass) in four equal portions with one of four diets, each with four repetitions for a period of 45 days. All diets contained 33% crude protein (isoprotein) and 355 kcal 100 gr^{-1} (isocaloric), with different percentages of sargassum powder: Diet "A" 15%; Diet "B" 10%, Diet "C" 5%, and Diet "D" without seaweed (control diet). Algae were collected, rinsed, dried and ground up for the feed formulations. Weight of the animals was measured at the beginning of the experiment and at 15-day intervals to assess their growth. The physicochemical variables of the water were measured every 2 days. Final biomass, biomass gain and specific growth rate (SGR) exhibited no significant differences between treatments ($P>0.05$). Survival rate was almost equal under the four experimental conditions, 95.2% to 97.00% ($P>0.05$). Diets "A" and "B", with a greater content of algae, exhibited better feed conversion (1.15:1 and 1.17:1) than diets "C" and "D" (1.30:1 and 1.33:1) ($P<0.05$). The physical-chemical variables of the water showed no significant variation and remained within the standards necessary for the wellbeing of the shrimps. If sufficient biomass of beached algae can be practically and economically collected, it may be used as a component in the making of shrimp feed.

Keywords: Oman Sea, *Sargassum*, Diet. Shrimp, Growth and Survival Rates

Introduction

There have been many studies on the use of seaweed in animal diets. For example, Cruz-Suárez et al. (2000) used *Macrocystis pyirifera* flour, and He and Lawrence (1993) used *Laminaria digitata* flour as a feed ingredient for the shrimp *Litopenaeus vannamei*. Tahil and Juinio-Menez(1999) used the seaweeds *Laurencia*, *Hypnea*, *Amphiroa* and *Coelothrix* as food for *Haliotis asinina* (Gastropoda). Seaweeds are rich in proteins, vitamins, carbohydrates, fiber, lipids and minerals. When fresh, they are 75–85%water and 15–25% organic components and minerals. Dry matter is 65–85% organic substances and 30–35% ash(Halperin 1971; FAO 2005). Some species of algae may contain greater contents of protein, carbohydrates and fat than the ingredients traditionally used in shrimp diets. According to Diaz-Peferrer and Lopéz (1961), marine algae possess all the essential minerals for animals.

The utilization of algae in the feeding of shimp should be possible and the aim of this study was to test whether seaweeds can be used as a source of protein in shrimp diets.

Materials and Methods

Experimental design

A total of 500 juvenile shrimp *L. vannameii* (3 grams) were used. Samples were acquired from a commercial shrimp farm and transported in plastic bags with oxygen to the Laboratory of Off-Shore Fisheries Research Center Chabahar- IRAN. In the laboratory, the animals were kept in a 5,000 L tank for 7 days with constant aeration and fed *ad libitum* with a commercial feed 35% protein content for acclimation to local conditions.

The juveniles were then starved for 24h prior to the beginning of the experimental phase. For the feeding experiments, the shrimps were kept in 16 plastic tanks (each with 300L water and 30 juvenile) for 45 days. The design was entirely randomized. Water was treated with activated carbon filters and aeration using two 3L min^{-1} air-pump in order to maintain stable physical-chemical conditions.

The tanks were siphoned daily to remove fecal matter, uneaten feed, molted exoskeletons and other organic wastes. Feeding (4% of total biomass, adjusted weekly) was carried out in four portions at a proportion of 40% in the morning and 60% in the afternoon. The laboratory was illuminated with fluorescent light, maintaining a 14:10 h light: dark photoperiod. During the experiments, oxygen, temperature, salinity and pH were measured every 2 days.

Formulation of diets

The diets were formulated as follows: seaweed flour (made up of the *sargassum*), 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).

The selection of this species for the processing of seaweed meal was based on a preliminary study undertaken over a 12-month period (Gharanjic et al., 2011). The dominant seaweed species from the Chabahar area of the beach of the Oman sea- Iran was collected and used as proportional feed for this experiment.

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 floura	13.0	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 permix[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, F 1.51, 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 kcalkg−1

(CP crude protein; DM dry matter; EE ether extract, F fiber, A Ash, 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 2000 IU kg−1 , niacin 2,500 mg,folic acid 80 mg, pantothenic acid 1,200 mg, selenium 25 mg

The total biomass of the dominant species was dried at 55°C for 36h and weighed. The seaweed meal, which was made in Havoorash shrimp feed factory- Bushehr, was ground to a fine powder in a hammer mill. In formulating the isoprotein and isocaloric diets, with33% crude protein and around 355 kcal 100 gr^{-1}, the pro- portions of components were calculated following procedures described by Correia (2004), EMBRAPA (1989) and the National Research Council (1989), and employed in the seaweed analyses (Tables 2, 3). The feed ingredients were ground to a powder, homogenized with 40% water at 60°C, placed in a meat mincer pellet former (2 mmdiameter) and then dried in an oven at 60°C for 24 h. The feed was conditioned in plastic containers and stored at room temperature. Feed conversion was determined by the amount of feed ingested divided by the weight gain of the shrimps. Survival rate was determined from the number of animals alive at the end of the experiment. Specific growth rate was calculated using the formula:

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

where P_f is thefinal weight, P_i is the initial weight, and t is time.

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

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})

Ingradients	***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
Moisture	16.11±1.00
Caloric Value	235.1 ±7.12

(a): calculated by100-(Crude Protein+Crude Fat +Ash+Carbohydrate)(AOAC, 1990)

Results and Discussion

The shrimps 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.

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

Growth was calculated for the 45 days of cultivation. Final biomass values ranged from 350.27 to 360.89 g tank^{-1};gains in biomass ranged from 259.50 to 270.56 g tank^{-1}, and SGR ranged from 3.04 to 3.09%. A statistical comparison between treatments revealed no significant differences (P>0.05). Therefore, the replacement of proteins of soy, fish and wheat flour with seaweed resulted in no interference with regard to growth. Survival rate ranged from 95.20% (Feed D) to 97.00 % (Feeds B and C). Comparatively, there were no statistical differences (P>0.05) and there was an average of 96.35% in the stock density of one juvenile per 10 liter over the 45-day period. Statistical analyses demonstrated that the treatments had no influence on shrimp survival rate. Feed conversion ranged from 1.15 to 1.33, with differences among the four treatments (P≤0.05), but treatment A and B were similar to one another, as were treatments C and D (Table 4). The averages of the physical-chemical variables of temperature: 27.44°C; salinity: 19.62; oxygen: 4.10 mg L^{-1}, and pH: 7.43.

Table 4. Growth 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≤0.05). SGR Specific growth rate

Data Treatment	**A**	**B**	**C**	**D**
Initial biomass (gr .tank^{-1})	90.10±0.08 ^{b}	90.42±0.15 ^{a}	91.78±0.15 ^{a}	90.77±2.12 ^{a}
Final biomass (gr. tank^{-1})	360.66±3.19 ^{a}	355.82±2.03 ^{a}	360.89±2.08 ^{a}	350.27±2.97 ^{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 (gr. tank^{-1})	270.56±3.11 ^{a}	265.40±1.97 ^{a}	268.22±2.72 ^{a}	259.50±3.15 ^{a}
Survival (%)	96.20±4.18 ^{a}	97.00±2.73 ^{a}	97.00±2.73 ^{a}	95.20±6.73 ^{a}
Feed conversion ratio^{c}	1.15±0.0 ^{b}	1.17±0.03 ^{b}	1.33±0.17^{b}	1.30±0.22 ^{b}
Cultivation periods (days)	45	45	45	45
Juvenile density /a300 tank	30	30	30	30

c Total feed supplied in dry weight /biomass gain in wet weight

Pedreschi-Neto (1999) obtained averages between 0.11 and 0.20g for final biomass, and 1.68 to 3.17% in specific growth rate using post-larvae for a period of 60 days, i.e.,well below the values obtained in the present study. Other experimental results from the same author showed a survival rate ranging between 51.7% and 60.0%, with anaverage of 55.35%, which were also well below the values obtained in the present study. On the other hand, data on feed conversion from this same experiment using popcorn residuals (Zea mays L.) in *Litopenaeus vannamei* juvenile feeds were between 1.23 and 1.81, which were little higher than those of the present study.

Cornejo *et al*. (1999) tested the effect of the seaweed *Caulerpa sertularioides* on the growth, survival and biomass of the brown shrimp *Penaeus californiensis* for a 10-week period in 150 L tanks with three repetitions of three treatments: Treatment one with no seaweed, but commercial feed with 35% crude protein; Treatment two in direct presence of seaweed with commercial feed; and Treatment three direct presence of seaweed with commercial feed. The results for growth, survival and production were the following: Treatment one, 0.46±0.4 g, 68.7±1.2% and 5.6±1,1 g; Treatment two, 0.73±0.4 g, 75±1.0% and 7.8±1.2 g; and Treatment three, 3.98±0.4 g, 100% and 36.24±4.3 g, respectively. The author concludes that the presence of the algae *C. sertularioides* has a direct effect on the growth, survival and biomass of the brown shrimp *P. californiensis* under laboratory conditions.

In analyzing the digestibility of nine commercial shrimp feeds in Mexico, Cruz-Suárez et al. (2000) obtained survival rates of 100% in 14 days for three treatments. At 28 days, survival ranged from 94% to 98%. Feed conversion using *Phaeophycea* algae flour was 2.63±0.42; 2.80±0.27 and3.12±0.54, using 0, 4% and 8%, respectively. These values were lower than those of the present experiment for survival. Two of the nine diets tested contained kelp flour Or*phyco colloids* in the formula. However, other parameters should be taken into consideration; for example: the cost of transport of raw materials and the storage structure, takinginto account the feed composition.

The physical-chemical variables remained within the range recommended for *L. vannamei* by Rocha et al.(1998), Clifford (1992), Barbieri *et al.* (2001), Álvarez *et al.* (2004) and McGraw and Scarpa (2004), such that these variables did not interfere with the treatments.

In conclusion, this study found that the marine seaweed *Sargassum illicifolium*is viable for use in the feeding of *L. vannamei*, with effect on shrimp growth rates. The results suggest that there is an increase in feed conversion when the levels of algae are increased. Also, this increase in the proportion of algae in the feed was associated with increased fishmeal levels. However, it is necessary to test the algae dissociated from the levels of fishmeal.

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