Dietary effects of seaweed *Sargassum ilicifolium* on reducing cholesterol level of white leg shrimp (*Litopenaeus vannamei)*

Hafezieh M.[1*]; Ajdehakosh A.[2]; Hosseini S.H.[2]

Received: June 2016 Accepted: December 2016

Abstract

In this research, the nutritional effects of *Sargassum ilicifolium* Chabahar Bay, Oman Sea, on cholesterol levels of white-leg shrimp (*Litopenaeus vannamei*) were studied. The seaweed was collected from coastal areas, rinsed, dried, powdered and the nutritional values were measured in the laboratory. A part of protein resources of shrimp feed replaced with seaweed powder in four treatments (D: as control without any replacement) C: with 5%, B: 10 % and A: 15% seaweed replacement, each with three replicates in order to obtain isonitrogenus 33% CP., and Isocaloric (13% fat and 15% carbohydrate) feed. Dried diets were used according to the daily need of shrimp, calculated after each 10 day biometry. Water stability and absorption capacity of the pellets in sea water were measured and compared statistically. Juvenile shrimps (Initial body weight=3 g) were acclimated for one week under hatchery conditions and were fed 3-5% of their body weight. Abiotic parameters, weight and length biometry were measured on day two and day 10, respectively. After 45 days final biometry, body analysis were measured, and muscle colorimeter were conducted using HPLC. There were no significant differences ($p>0.05$) between body lipid among treatments, but cholesterol content (mg/100gWW) showed significant differences ($p<0.05$), the lowest (121.68±12.12) was in treatment A, and the highest in D (147.92±11.02). Treatments A and B showed color changes to pink- partial orange and pink in shrimp muscle with no differences compared to white and no color in shrimp in treatments C and D. It seems that this color change plays a major role in market acceptability.

Keyword: Cholesterol, Color, Feed, White-leg shrimp, Seaweed

1-Iranian Fisheries Science Research Institute (IFSRI), Agricultural Research Education and Extension Organization (AREEO), P.O. Box: 14965/149. Tehran, Iran.
2-Off-shore Fisheries Research Center, (IFSRI), Agricultural Research Education and Extension Organization (AREEO), Chabahar, Iran.
*****Corresponding author's Email:jhafezieh@yahoo.com

Introduction

There have been many studies using seaweed in animal diets. He and Lawrence (1993) used *Laminaria digitata* and Cruz- Suárez *et al.* (2000) used *Macrocystis pyirifera* flour as a feed ingredient for the shrimp *Litopenaeus vannamei*. Tahil and Juinio- Menez (1999) used the seaweeds *Laurencia, Hypnea, Amphiroa* and *Coelothrix* as feed 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. Drymatter 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 feeding shrimp 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 to reduce the cholesterol level of shrimp.

Materials and methods

Experimental design

A total of 500 juvenile shrimp (3 g each) 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 to acclimate to local conditions.

The juveniles were then starved for 24 h prior to thebeginning of the experimental phase. For the feeding experiments, the shrimp were kept in 16 plastic tanks (each with 300 L water and 30 juvenile) for 45 days. The design was entirely randomized. Water was treated with activated carbon filters and aeration using two 3 L min^{-1} airpumps 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 treatments 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 to contain: algae flour (made up of 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 flour (Table 1).

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 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, EE16.00, 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, 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, vitamin B6 300 mg, vitamin B12 1, 200 mg, E 2000 IU kg^{-1} , niacin 2500 mg, folic acid 80 mg, pantothenic acid 1200 mg, selenium 25 mg.

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.

The total biomass of the dominant species was dried at 55°C for 36 h and weighed. The seaweed meal, which was made in Havoorash shrimp, feed factory-Bushehr, was ground to a finepowder in a hammer mill. In formulating the isoprotein and isocaloric diets, with 33% crude protein and around 355 kcal 100 g^{-1}, the proportions 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 and 3). The feed ingredients were ground to a powder, homogenized with 40% water at 60°C, placed in a meat mincer pellet former (2 mm diameter) 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 shrimp. Survival rate was determined from the number of animals alive at the end of the experiment. Proximate and chemical composition such as cholesterol level of shrimp flesh was analyzed using standard methods (Cruz- Suarez *et al.*, 2008b).

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 compositions 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

After 45 days and the final biometry, body analyses, and muscle colorimetric measurement were done with HPLC. The data obtained were tested with pp Plot to determine the parametric data and statistical differences were detected using one–way ANOVA, Duncan test of SPSS software.

Results

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: A=15% seaweed +7% soy flour, 37% fish flour and 13% wheat flour, B=10% seaweed +12% soy flour, 33% fish flour and 19% wheat flour; C=5% seaweed+16%soy flour, 30% fish flour and 25% wheat flour; and D=0% seaweed +22%soy flour, 26% fish flour and 27% wheat flour.

Growth was calculated for the 45 days of cultivation. Final biomass values ranged from 300.89 to 334.82 g m^{-2}; gains in biomass ranged from 210.49 to 215.36 g m^{-2}, and SGR ranged from 4.68 to 5.68%. 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 ($p \leq 0.05$), but treatment A and B were similar to one another, as were treatments C and D (Table 4). Cholesterol content of body were significantly differences between treatment using seaweed powder compared to the control ($p<0.05$). The lowest cholesterol content was measured in treatment A (121.68±12.12) and the highest was in treatment D (147.2±11.01). Using the seaweed in shrimp feed ingredients changed the body color from pink to orange (3 in A and B, not sig. $p>0.05$) significantly compared to that in treatments C (2) and D (1) ($p<0.05$). The numbers between 1 to 3 means white (1), pink (2) and pink to orange (3).The averages of the physical-chemical variables were: 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±SD, n=100 per treatment group). Different letters on the same line indicate statistical differences ($p \leq 0.05$). SGR (Specific Growth Rate).

Data Treatment	A	B	C	D
Initial biomass (g L^{-1})	11.10±0.08 ^{a}	11.42±0.15^{a}	11.78±0.15^{a}	11.77±2.12^{a}
Final biomass (g L^{-1})	128.66±3.19^{a}	134.82±2.03^{a}	120.89±2.08^{a}	121.27±2.97^{a}
SGR (%/day)	5.65±2.85^{a}	5.68±1.81^{a}	5.17±1.86^{a}	4.68±2.65^{a}
Gain in biomass (g L^{-1})	118.56±3.11^{a}	124.36±1.97^{a}	109.12±2.72^{a}	106.49±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*	1.15±0.0^{b}	1.17±0.03^{b}	1.33±0.17^{a}	1.30±0.22^{a}
Cholesterol (mg. 100g WW)	121.68±12.12^{a}	127.54±14.33^{b}	130.84±10.25^{c}	147.2±11.01^{d}
Colorimetric **	3^{c}	3^{c}	2^{b}	1^{a}
Cultivation periods (days)	45	45	45	45
Juvenile density /a300 tank	30	30	30	30

* Total feed supplied in dry weight /biomass gain in wet weight.
**Colorimeter between 1-3 mean 1 for white color, 2 for pink, 3 for pink to orange.

Discussion

Pedreschi-Neto (1999) obtained averages between 0.11 and 0.20 g 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 an average 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 *L. 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 *Caulerpasertu larioides* on growth, survival and biomass of the brown shrimp *Penaeus californiensis* for a 10 week period in 150 L tanks with three treatments each with three repetitions: Treatment 1—

with no seaweed, but commercial feed with 35% crude protein; Treatment 2—indirect presence of seaweed with commercial feed; and Treatment 3—direct presence of seaweed with commercial feed. The results for growth, survival and production were as follows: Treatment 1, 0.46±0.4 g, 68.7±1.2% and 5.6±1, 1 g, respectively; Treatment 2, 0.73±0.4 g, 75±1.0% and 7.8±1.2 g, respectively; and Treatment 3, 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 and 3.12±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 *phycocolloids* in the formula. However, other parameters should be taken into consideration; for example: the cost of transport of raw materials and the storage structure, taking into account the feed composition.

Similar to body color, cholesterol and lipid carcass composition of shrimp can be reduced and changed with consumption of *Sargassum* meal (Casas- Valdez *et al.,* 2006) and *Ulva clatheata* (Cruz- Suarez *et al.,* 2008a). *Ulava* is known to change fat deposition and mobilization patterns in sea bream (Nakagawa *et al*., 1987), apparently resulting in a more efficient use of fat depositions 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 salts, at least in sea bream (Une *et al*., 1991), and this may be the basis for its effects on fat and cholesterol metabolism. Conceivably, animals naturally adapted to diets that include cysteinolic acid require it for efficient fat metabolism. Cysteinolic acid could play other roles in metabolism as well. For example, it could be converted to cycteine (Cruz- Suarez *et al*., 2008b).

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

In conclusion, this study found the marine algae *Sargassum ilicifolium*is viable for use in the feeding of *L. vannamei*, with effects on shrimp growth rates and reduced cholesterol levels.

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.

Acknowledgments

Many thanks of Havoorash shrimp feed factory for supplying the ingredients and making the diets. Also thanks to Iranian Fisheries Science Research Institute (IFSRI) for the financial support.

References

Álvarez, A.L., Racotta, I.S., Arjona, O. and Palacios, E., 2004. Salinity stress test as a predictor of survival during grow out in Pacific's white shrimp (*Litopenaeus vannamei*). *Aquaculture*, 237, 237–249

Casas- Valdez, M., Portillo-Clark, G., Aguila-Ramirez, N., Rodriguez-Astudillo, S., Sánchez-Rodríguez, I., Carrillo- Dominguez, S., 2006. Efecto del alga marina Sargassum spp. Sobre las variables productives y la concentracion de colestrol en el camaron café, Farfantepenaeus californiensis (Holmes, 1900). *Revista de Biología Marina y Oceanografía Journal*, 41(**1**), 97-105.

Clifford, H.C., 1992. Marine shrimp pond management: a review. In: Chamberlain, G.W., Villalón, J. and Wyban, J., (eds) Proceeding of special session on shrimp farming, 1992. World Aquaculture Society, Orlando, pp. 110–137.

Cornejo, M.A.P., Curdova, L.M., Barajas, F.M., Pramo, J.N. and Clark, G.P., 1999. Efeito de la macroalga *Caulerpasertu larioides* en el desarrollo delcamarón *Penaeus californiensis* (Decapoda: Peneidae). Centro de Investigaciones Biológicasdel Noroeste, Sonora.In: Robson Liberal da Silva and José Milton Barbosa, 2009.

Correia, E.S., Silva-Junior, R.F., Nono, R.G.B. and Santos, R.M.S., 2004. Avaliação do crescimento do camarãomarinho (*Litopenaeus vannamei*) e da qualidade da águaemfunção da utilização dequatro raçõescomerciais. VI Simpósio Brasileiro de Criadores deCamarão, 2004, Natal.In: Robson Liberal da Silva & José Milton Barbosa, 2009.

Cruz-Suárez, L.E., Rieque-Marie, D., Tapia-Salazar, M. and Guajardo-Barbosa, C., 2000. Uso de harina de kelp (*Macrocystis pyrifera*) en alimentos paracamarón. Avancesen Nutrición Acuicola. V Simposium Internacional de Nutrición Acuícola, 2000.Centro de Investigaciones y de Estudios Avanzados, Mérida,pp 1–40. In: Robson Liberal da Silva and José Milton Barbosa, 2009.

Cruz- Suarez, L.E., Leon, A.A., Pena-Rodriguez, A., Rodriguez-Pena, G., Moll, B., Ricque-Marie, D., 2008a. Shrimp and green algae co-culture to optimize commercial feed utilization. ISNF XIII international symposium on nutrition and feeding in fish Florianopolis, June 1 to 5, Brazil.

Cruz- Suarez, L.E., Tapia- Salazar, M., Nieto- Lopez, M.G. and Ricque- Marie, D., 2008b. A review

of the effects of macro algae in shrimp feeds an in Co- culture, pp. 304-333. Editors: Cruz- Suarez, L.E., Tapia- Salazar, M., Nieto-Lopez, M.G. and Ricque- Marie, D., Villarreal Cavazos, A.D., Lazo, J.P. and Teresa Viana, Y. M., Advances en Nutricion Acuicola IX. IX Simposium International de Nutricion Acuicola. 24-27 Noviembre. Universidad Autonoma de Nuevo Leon, Monterrey, Nuevo Leon, Mexico.

Diaz-Peferrer, E. and Lopéz, H., 1961. Taxonomia, ecologia y valornutrimental de algas marinas cubanas. Instituto Cubano deI nvestigación Tecnológica, Serie Estudios Sobre Trabajos deInvestigación, Habana. In: Robson Liberal da Silva and José Milton Barbosa, 2009.

EMBRAPA, 1989. Tábua de composiçãoquímica e valoresenergéticos De alimentos parasuínos e aves. Centro Nacional de Pesquisa de Suínos e Aves, ConcórdiaIn: Robson Liberal da Silva and José Milton Barbosa, 2009.

FAO, 2005. Aquaculture production statistics. Available at: http:/www.fao.org. Accessed on 24 October 24, 2005.

Gharanjic, B.M., Azhdari, D., Azini, M.R. and Balooch, G.M., 2011. Estimated of seaweed in beach of Oman Sea- Chabahar. Final report of Research Project. Iranian Fisheries Research Organization.175P.

Halperin, D.R., 1971. Las algas en la alimentación humana. ContribuciónTécnica n. 10. CIBIMA, Buenos Aires.

He, H. and Lawrence, A.L., 1993. Vitamin C requirements of the shrimp *Penaeus vannamei*. *Aquaculture*, 114, 305–316. Doi: 10.1016/0044-8486(93)90305-I. Maturação e Larvicultura. Aprenda Fácil, Viçosa. In: Robson Liberal da Silva and José Milton Barbosa, 2009.

McGraw, W.J. and Scarpa, J., 2004. Mortality of freshwater-acclimated *Litopenaeus vannamei* associated with acclimation rate, habituation period, and ionic challenge. *Aquaculture*, 236, 285–296. Doi:10.1016/j.aquaculture.2004.01.037.

Nakagawa, H., Kasahara, S. and Sugiyama, T., 1987. Usefulness of Ascophyllum meal as feed additive for red sea bream, *Pagrus mahor*. *Aquaculture*, 151, 275- 281.

National Research Council, 1989, Nutrient requirements of warm water fishes and shellfishes. National Academy, Washington.

Pedreschi- Neto, O., 1999. Utilização de resíduos do milho de pipoca (Zea mays L.) emraçõesparapós-larvas do camarão *Litopenaeus vannamei* (Boone, 1931). Dissertation, Federal University of Pernambuco.

Rocha, M.R.M., Nunes, M.L. and Figueiredo, M.J., 1998. Cultivo de pós-larvasde *Penaeus vannamei*e mberçários intensivos. *Anais Aqüicultura Brasil*, 98, 298–299.

Tahil, A.S. and Juinio- Menez, M.A., 1999. Natural diet, feeding periodicity and functional response to food density of the abalone, *Haliotisas inina* L., (Gastropoda). *Aquaculture Research*, 30, 95–107.

Une, M., Goto, T., Kihira, K., Kuramoto, T., Hagiwara, K., Nakajima, T. and Hoshita, T., 1991. Isolation and identification of bile salts conjugated with cysteinolic acid from bile of the red sea bream, *Pagrosomus major*. *Journal of Lipid Research*, 32, 1619- 1623.