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RESEARCH ARTICLE

NUTRITIONAL ENHANCEMENT OF *ARTEMIA PARTHENOGENETICA* USING *SARGASSUM WIGHTII* AS DIETARY SUPPLEMENT

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ABSTRACT

Three replicas of 5 glass bowls containing seawater medium were inoculated with *Artemia parthenogenetica* at the concentration of 1000 instar I nauplii and the 1st bowl was fed with rice bran alone (control) while the 2nd , 3rd, 4th, and 5th bowls were fed with rice bran as well as 250mg, 500mg, 750mg and 1000mg *Sargassum* powder respectively. On 35th day, *Artemia* were collected, dried and analyzed for nutritional enhancement. *Sargassum* powder provides some extra amounts of crude proteins, dietary fibers, essential amino acids, long chain fatty acids, minerals and vitamins in addition to the normal amounts supplied by rice bran. Hence, the concentration of these nutritional components increased with increase in the dosage of *Sargassum* powder up to 500mg/day but higher dosages (750mg and 1000mg) do not influence the nutrients enhancement due to the constrain of feed intake of *Artemia*. The correlation coefficients of increase in the total essential amino acids were r= +23 (P<0.05) at 250mg dosage, r= +27 (p>0.05) at 500mg dosage, r= +26.2 (p<0.05) at 750 mg dosage and r= +26.1 (p>0.005) at 1000 mg dosage of *Sargassum* powder in relation to the values in the control. The correlation coefficients of increase in the total fatty acids were r= +21 (P<0.05) at 250mg dosage, r= +26 (p<0.05) at 500mg dosage, r= +24 (p>0.05) at 750 mg dosage and r= +24.1 (p>0.005) at 1000 mg dosage of *Sargassum* powder per day. This study therefore recommends the growers to use the maximum of 500mg *Sargassum* as a feed supplement to *Artemia* for nutritional enrichment.

INTRODUCTION

Nutritional enhancement of *Artemia* has been one of the latest areas of research and development, which attracts many workers to promote the growth, survival and reproductive capabilities of *Artemia* for having more biomass production and to enrich *Artemia* with nutritionally valuable fatty acids and amino acids necessary for growth and performance of aquaculture animals. In hatcheries, nauplii have been enriched by feeding the nauplii with Menhaden oil, SELCO, Algamat-2000, Micro Feast L-10 and DHA SELCO which enable the nauplii to accumulate long chain polyunsaturated fatty acids required for the growth of young fish fingerlings and other aquatic animals (Tamaru *et al*, 2003). Fulk and Main (1991) enriched *Artemia* by growing it along with microalgae as its feed and observed the nutritive value for growing fishes. Tamaru et al (1993) grew nauplii of Artemia using baker's yeast as its feed and used the enriched nauplii for growing stripped mullets (*Mugil cephalus*). Hilda (1992) evaluated the suitable concentration of *Reprostim* for the promotion of survival, growth and reproduction in *A. franciscana*. Devi (1995) found out the efficacy of the Ayurvedic product *Asparagus racemosus* on cyst induction in *A.franciscana* by growing it in seawater medium containing different concentration of that drug. Prema and Palavesam (2004) evaluated some mono and mixed ayurvedic drugs for boosting the survival, growth and reproductive capabilities of *A. parthenogenetica*. Mony (1998) studied the use of some ayurvedic drugs in improving the reproductive performance in *A. parthenogenetica* from Thamaraikulam salt pan, South India. Tamaru *et al.* (1999) fed *Artemia* nauplii with oils of some plant and animal sources and studied their fatty acids profiles in it to improve the biochemical compositions and nutritional quality. Citarasu *et al.* (2002) fed *Artemia* with herbal diets *Hygrophila spinosa, Withania somnifera, Zingiber officinalis*, *Solanum trilobatum, Andrographis paniculata* and *Psoralea corylifolia*, and found out that they promote growth and survival, and improve biochemical composition of *Artemia*. Immanuel *et al.* (2004) enriched *Artemia* with highly unsaturated fatty acids (HUFA) and fed tiger shrimp to observe growth and survival of the shrimp. Michel Babu *et al.* (2006) enriched *Artemia* with *Zingiber officinalis* which is an appetizer to stimulate fast feeding and growth, and analyzed its biochemical composition in order to improve the nutritional quality for high productivity in tiger shrimp. Rekha et al (2007) enriched *Artemia salina* nauplii with microalgae and baker's yeast for enhancing the nutritional value to use in laviculture. Michael Babu *et al.* (2008) enriched *Artemia* with extracts of terrestrial herbs *Withania somnifera* and *Mucuna*

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pruriens to improve its biochemical contents to boost up the growth of tiger shrimp (*Penaeus monodon*). Ben Naceur *et al.* (2008) characterized the biological aspects of *A. salina* population by analyzing cyst formation, lipids, essential fatty acids and reproductive characteristics. The brown seaweed *S. wightii* is a macroscopic marine alga found attached to rocky bottom of shallow coastal waters of Tamilnadu and many parts of Asia, which has been used as animal feed, food ingredients, fertilizer, medicines and raw materials (Levering *et al*., 1969). This marine alga is a good source of minerals, vitamins (A, B_1, B_2, C, D and E), proteins, essential amino acids, fats, fatty acids, polysaccharides, fibers and flavanoids (Lahaye, 1991 and Darcy-Vrillon, 1993) and its protein and lipid qualities are acceptable for humans and veterinary animals as it contains relatively high proportion of essential amino acids and unsaturated fatty acids (Thillaikkanu Thinakaran *et al*., 2012). Dietary supplementation of *S. wightii* has enhanced the weight gain of mice (Yung-Choon Yoo *et al*., 2007), tiger shrimp (Felix *et al*., 2004.2005), ducks (Breikaa, 1993) and poultry (El-Deek *et al.* 2011). As this algal powder is rich in acceptable proteins, it has been recommended as a dietary supplement for cattle in China, Thailand, Korea, Japan, Indonesia and Philippines (Kolanjinathan *et al*., 2014). Therefore, *Sargassum* powder would enrich the *Artemia* with many nutritional components. The purpose of this study is to determine the effectiveness of using *Sargassum wightii* powder as a dietary supplement for enhancing the total proteins, lipids, essential amino acids and fatty acids of *Artemia* to support the fast growth of fishes and aquatic animals.

MATERIALS AND METHODS

Materials

For this *in vitro* culture, the starting materials called cysts of *A. parthenogenetica* were obtained from the salt pans of Tuticorin located on 8º 47' Northern latitude and 77º 68' Eastern longitude, and used in this study. Rice bran was purchased from local markets in Nagercoil and used as a feed to *Artemia*. This feed contained 3.40% moisture, 7.0% protein, 1.59% lipids, 53.06% carbohydrate and 5.91% free amino acids in its dry weight and is in convenient size that can be consumed by both nauplii and adults of *Artemia*. Seawater was collected from the Indian Ocean near Sanguthurai beach of Kanyakumari district and used as medium for the laboratory culture of the *Artemia* strain. The physico-chemical parameters of the seawater are given hereunder:

Salinity	-32.3ppm
pH	- 8.13
Light penetration	- 0.9m
Dissolved oxygen	- 2.65ml/L
Temperature	- 28.5 ºC (day time)
	- 23.2 ºC (night time)
Total phosphorus	- 2.37µgm/L
Nitrates	- 6.43 µgm/L
Nitrite	- 0.62 µgm/L
Primary productivity	-154mg c/m^3/hr.

Methods

Preparation of *Sargassum* powder: Specimens of *S. wightii* (Phaeophyceae) were collected from the coastal village Leepuram near Kanyakumari (Lat 9º11′ N; Long79º 24′E) of Tamilnadu and brought to the laboratory. They were washed repeatedly with tap water for 3 times to remove dust particles, sand and epiphytic microalgae. The whole plants were dried under shade, and then sun dried and ground into *Sargassum* powder. This powder was then stored in a refrigerator.

Chemical analysis of *Sargassum* powder and Rice bran

The protein content of *Sargassum* powder was estimated using the Biurrette method described by Raymont *et al.* (1964). The lipid was extracted using chloroform methanol mixture as a solvent and estimated using the method described by Folch *et al.* (1956). The method described in the AOAC (1995) was followed for the quantification of minerals in *Sargassum* powder. A 0.2 g of oven dried *Sargassum* powder was taken in a dry conical flask and treated with 10 ml of diacid mixture (2:5 of Nitric acid and Perchloric acid). The content of conical flask was allowed to stand for a few hours for cold digestion. After that, the conical flask was kept on a hot plate to digest the contents under the influence of temperature. The digested content was filtered through a Whatman No.40 filter paper to get a filtrate. The filtrate was suitably diluted and fed into ICP - Perkin Elmer Mayer Optical Emission Spectrophotometer (Optima 2100 DV) as per the procedure given in the Users' Manual for analyzing the amount of Mg, Cu, Mn, Fe and Zn present in the filtrate. Na, K, I and Ca were analyzed with Flame Photometer. For the estimation of essential amino acids, one gram of *Sargassum* powder was hydrolyzed with 6N hydrochloric acid in evacuated sealed tube for 24 hours at 110ºC and the hydrolyzed sample was analyzed with Waters Pico-Tag HPLC Amino acid Analysis System (Column: Pico-Tag amino acid analysis column, 3.9 (150 mm); detector: Waters 2489 Dual λ absorbance detector). The same methods were used for the rice bran also.

Preparation of Nauplii

The cysts of *A. parthenogenetica* were decapsulated chemically by chlorine bleach treatment described by Granvil D Treece (2000). About 1gram of cysts was incubated in 15ml of freshwater at room temperature for 1hour and the hydrated cysts were concentrated and poured into a chilled decapsulation solution containing 10ml of chlorine bleach (NaOCl; 5.5%) and 5ml of sodium hydroxide (NaOH; 40%) and incubated at 10ºC for 5 minutes. After decapsulation, 3.5g of sodium thiosulfate ($Na_2S_2O_3$) was added to the decapsulation vessel to neutralize the chlorine. A 250ml of seawater was taken in a conical flask and its pH was adjusted to 8.0 by adding 1.0% sodium bicarbonate solution; the flask was gently warmed to 28ºC±1ºC to assist in the hatching process. About 500mg of decapsulated *Artemia* cysts was added into the flask containing seawater and the flask was exposed to 1000 Lux light per day using a fluorescent tube light. Continuous aeration was given to the bottom of the flask at the rate of 10 to 20 lit. per minute. After 24 hrs of incubation, young nauplii were collected by scooping the top of the medium using a 100µm harvesting mesh bag as done by Lavens and Sorgeloos (1987). The instar I nauplii in the medium were counted by diluting 1ml of sample with 99 ml of seawater so as to have 1000 instars/ 1ml of medium.

Culture of *Artemia*

A 1.5 lit of seawater was taken in a large glass vessel and its pH was adjusted to 8.0 by adding 1.0% sodium bicarbonate

solution and slightly warmed to 28ºC±1ºC to provide optimum growth temperature. Triplicates of 5 bowls of 100ml capacity were cleaned well, filled with 100ml of the seawater in each bowl. 1 ml of medium containing about 1000 instar I nauplii was inoculated in each of the bowls which were then kept on a clear table in a room fitted with a fluorescent tube light capable of illuminating 1000 Lux light. Continuous aeration was given to the bottom of the bowls at the rate of 10-20 lit per minute to maintain proper level of dissolved oxygen in the medium. A 100gm of rice bran was dissolved in 200ml of water to get a feed suspension and 1ml of that feed solution was provided to the first bowl (control) daily, once in the morning and evening. A 100gram of *Sargassum* powder was dissolved in 200ml of water and the bowls were supplied with the following feeds both in the morning and evening:

Bowl I -500mg rice bran (1ml feed solution)
Bowl II -Rice bran +125mg Sargassum powder
Bowl III -Rice bran +250mg Sargassum powder
Bowl IV -Rice bran +375mg Sargassum powder
Bowl V -Rice bran +500mg Sargassum powder

Estimation of proteins

Artemia biomasses was collected by scoping with a 150µm net bag and dried on Whatmann filter paper. 2 gram of *Artemia* was used to estimate the protein content (%) by employing Lowry method (Lowry *et al*., 1951).

Estimation of lipid

Lipid was extracted from the *Artemia* samples by following the method of Folch *et al.* (1957) and its quantity in those samples was estimated by using the method of Barnes and Blackstock (1973).

Estimation of amino acids profile

Profile of free amino acids in *Artemia* was analyzed with the high performance liquid chromatography (HPLC) using the instrument LACHROM L-7000 according to the method of Suresh Babu *et al.* (2002). Methods described in the standard manual were followed for quantitative estimation of essential amino acids. The amino acids were detected based on the retention time established for the individual amino acid under defined experimental conditions while their concentrations were analyzed from the linearity of the peak areas for different concentrations, which was of course done by the HPLC system manager.

Estimation of fatty acids profile

Lipids were extracted using the method by Folch *et al.* (1957), and transmethylated overnight (Christie, 1982) after the addition of C19:0 (99% pure, Sigma Chemical Co., Poole, Dorest, UK) as an internal standard. Methyl esters were extracted with hexane: diethyl ether (1:1, v/v), and purified by thin layer chromatography (Silica Gel G 60, 20 X 20 plates, Merck) using hexane: ether: acetic acid (85:15:1.5, v/v/v) as solvent. The analyses of the methyl esters were performed on a PACKARD gas chromatograph (Packard Instrument Inc., Caversham, UK) equipped with a fused silica 50 m x 0.22 mm, an open tubular column coated with FFAP (film thickness: 0.25µm, SGE, UK, Ltd., London) and, an on-column injection system, using hydrogen as a carrier gas and a thermal gradient from 50 to 235°C. Peaks were recorded in a Shimadzu C-R 6 A Chromatopac recording integrator, identified by comparison with known standards, and quantified by means of the response factor of the internal standard.

RESULTS

Proximate Concentration of Nutrients

Chemical analysis shows that 1 gram of rice bran (basic feed) contains crude proteins (7.0%), crude fats (1.6%), dietary fibres (12.5%), essential amino acids such as cystine (7.9mg), histidine (4.1mg), leucine (10.4mg), isoleucine (6.4mg), methionine (2.8mg), phenylalanine (5.7mg), tryptophan (2.8mg), tyrosine (5.4mg), and valaine (8,2mg), minerals like calcium (1.0mg), phosphate (16mg), chloride (0.7mg), sodium (0.7mg), manganese (0.124mg), zinc (0.057mg), potassium (0.017mg), saturated fatty acids (7.4mg), monounsaturated fatty acids (6.6mg) and polyunsaturated fatty acids (9.5mg). Likewise, 1 gram of *Sargassum* powder contains crude proteins (12.3%), crude fats (3.4%), dietary fibres (32.2%), essential amino acids such as cystine (2.5mg), histidine (4.5mg), leucine (5.5mg), isoleucine (5.6mg), methionine (5.2mg), phenylalanine (3.7mg), tryptophan (6.4mg), tyrosine (5.3mg), and valaine (5.0 mg), minerals like calcium (67.2mg), phosphate (45.6mg), chloride (23.2mg), sodium (45.1mg), manganese (5.38mg), zinc (1.81mg), potassium (61.3mg), saturated fatty acids (11.7mg), monounsaturated fatty acids (9.2mg) and polyunsaturated fatty acids (12.20mg). Proximate analysis shows that concentration of all the nutritional components increased with increasing dosages of *Sargassum* powder from 250mg to 1000mg (Table-1). Highest concentration of these nutrition components was found at 1000mg, followed by 750mg, 500mg, 250mg and control in a descending order. Dietary components in all the *Sargassum* treatments were higher than those in the control (rice bran).

Protein Content

The protein content of *A. parthenogenetica* in response to different feed levels is illustrated in figure-1. In *Artemia* obtained from the control which was fed only with rice bran, the total protein content was 52.50±1.20%. In *Artemia* fed with rice bran and 250mg *Sargassum*/day, the protein content was 54.16±1.06%. In *Artemia* fed with rice bran and 500mg *Sargassum*/day, it was 56.85±1.02% while in *Artemia* fed with rice bran and 750mg *Sargassum*/day, it was 57.14±1.56%. The 1000mg dosage of *Sargassum* did not give considerable variation from 750mg dosage. The total protein content increases with increase in the dosage of *Sargassum* powder from 250mg to 750mg and there was no more increase in protein content while increasing the dosage further. The statistical significance of variance was $p<0.05$.

Lipid content

Figure-2 shows the differences in the lipids content of *Artemia* fed with rice bran and different gradations of *Sargassum* powder. In the control, the total protein content was 13.40±1.18% and it increased from 15.20±1.69% (at 250mg *Sargassum*) to 16.94±1.36% (at 750mg *Sargassum*) and then there was a slight decrease (16.53±1.36% at 1000mg *Sargassum*). The maximum lipid content was noted at 750mg *Sargassum* powder. The statistical significance of variance was $p<0.05$.

Table 1. Proximate concentration of various nutritional components in the experimental feeds

Chemical components	Rice bran (1000 mg)	Addition of Sargassum powder/day			
		250 mg	500mg	750 mg	1000mg
Crude protein %	7.0	10.1	13.2	16.3	19.4
Crude lipids %	1.6	2.4	3.3	4.2	5.0
Dietary fibers %	12.5	20.6	29.1	37.6	46.1
Amino acids					
Cystine (mg)	7.9	8.5	9.3	9.9	10.5
Histidine (mg)	4.1	5.2	6.3	7.4	8.5
Leucine (mg)	10.4	11.7	13.1	14.4	15.6
Isoleucine (mg)	6.4	7.8	9.2	10.6	11.0
Lycine (mg)	7.8	9.1	10.4	11.7	13.1
Methionine (mg)	2.8	3.7	4.5	5.3	6.2
Phenylalanine	5.7	7.3	8.9	10.5	11.9
Tryptophan (mg)	2.8	3.5	4.1	4.7	5.3
Tyrosine (mg)	5.4	6.7	7.0	8.1	9.2
Valine (mg)	8.2	9.5	11.3	12.7	13.8
Minerals					
Calcium (mg)	1.0	17.8	41.8	58.6	77.2
Phosphate (mg)	16	27.4	41.6	57.4	71.0
Chloride (mg)	0.7	6.5	12.3	18.2	23.9
Sodium (mg)	0.7	11.9	23.2	34.8	35.8
Manganese (mg)	0.124	1.7	2.9	4.4	5.6
Zinc (mg)	0.057	0.51	1.12	1.67	2.97
Potassium (mg)	0.017	15.8	31.6	46.2	63.3
Fatty acids					
Saturated fatty acids (mg)	7.4	10.3	13.2	15.4	16.6
Monounsaturated fatty acids (mg)	6.6	8.9	11.2	13.4	15.6
Polyunsaturated fatty acids (mg)	9.5	12.0	15.1	18.3	21.5

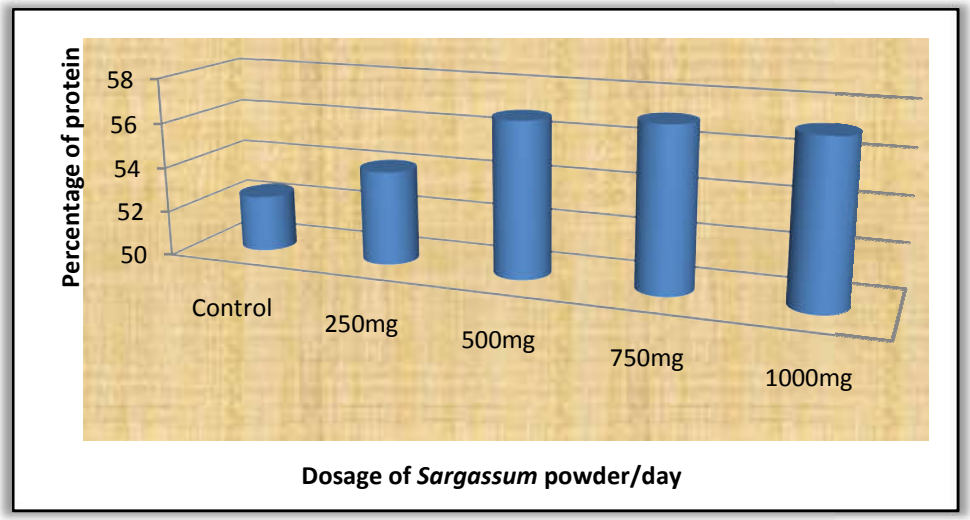

Figure 1. Percentage of proteins in the dry weight of *A.parthenogenetica*

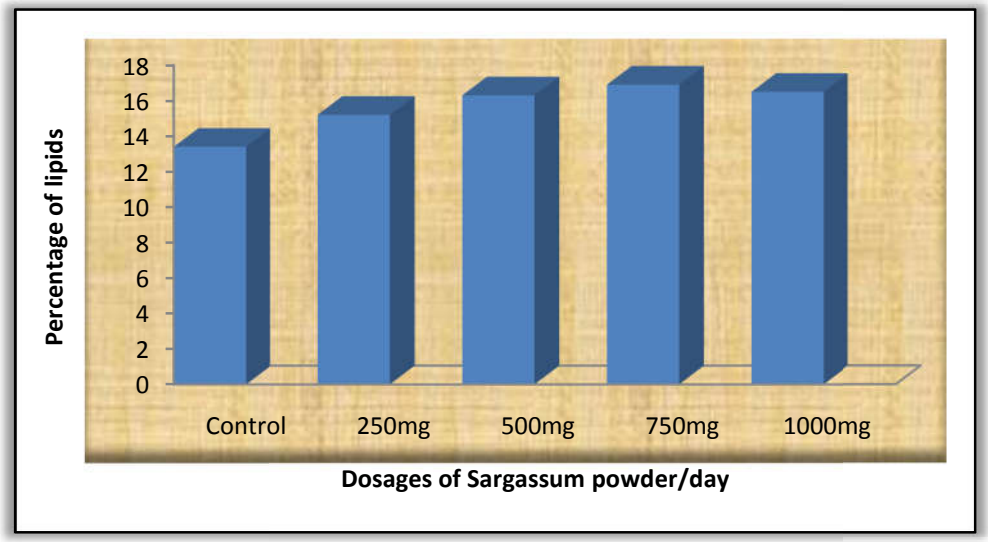

Figure 2. Percentage of lipids in dry *Artemia parthenogenetica*

Table 2. Concentrations of essential amino acids (mg/g) in the *Artemia* fed with rice bran and with rice bran plus 250mg, 500mg, 750mg and 1000mg *Sargassum* powder

Amino acid (mg^1gram)	Rice bran (1000 mg)	Addition of *Sargassum* powder/day			
		250 mg	500mg	750 mg	1000mg
Cystine	7.32	8.25	9.39	9.92	9.90
Histidine	17.61	19.57	21.38	21.41	21.39
Leucine	24.24	26.72	29.13	29.21	29.20
Isoleucine	16.42	17.81	19.32	19.36	19.30
Lysine	40.28	42.91	45.24	45.28	45.25
Methionine	9.21	10.51	11.38	11.93	11.42
Phenylalanine	14.73	16.23	17.92	17.75	17.71
Tryptophan	2.81	3.43	3.97	3.92	3.87
Tyrosine	14.41	15.53	16.70	16.69	16.62
Valine	13.40	14.61	15.74	15.37	15.32

Table 3. Concentrations of fatty acids (mg/g) in the *Artemia* fed with rice bran and with rice bran plus 250mg, 500mg, 750mg and 1000mg *Sargassum* powder

Fatty acid (mg^{-1}gram)	Rice bran (1000 mg)	Addition of *Sargassum* powder/day			
		250 mg	500mg	750 mg	1000mg
Palmitic acid (C16:0)	1.67	1.85	1.93	1.92	1.90
Stearic acid (C18:0) Aracchidic acid (C20:0)	0.42	0.67	0.83	0.82	0.82
Palmitoleic acid (C16:1)	0.13	0.32	0.45	0.46	0.48
Oleic acid (C18:1)	1.23	1.41	1.52	1.46	1.46
Linoleic acid (C 18:2)	2.41	2.91	3.04	2.98	2.99
Linolenic acid (C18:3)	0.49	0.51	0.59	5.58	5.58
Omega-3-fatty acid (C20:5)EPA Omega -3-fatty acid DHA (C22:6)	0.46	0.53	0.59	0.62	0.71
	0.86	0.94	1.37	1.42	1.43
	0.23	0.34	0.44	0.47	0.49

Table-2 clearly shows the differences in the lipids content of *Artemia* fed with rice bran and different gradations of *Sargassum* powder along with rice bran. In the control, cystine content was 7.32mg/g of *Aremia* and it increased from 8.25 to 9.95mg/g while increasing the dosages of *Sargassum* from 250mg to 1000mg. The maximum cystine content was noted at 750mg *Sargassum* powder. In the control, histidine level was 17.61mg/g of Aremia and it increased further from 19.57 to 21.41mg/g while increasing the dosages of *Sargassum* from 250mg to 750mg. The maximum histidine level content was noted at 750mg *Sargassum* powder. Leucine level in the control *Artemia* was 24.24mg/g and it increased from 26.72 to 29.21mg/g in *Sargasssum* fed Aremia. The maximum leucine level was noted at 750mg *Sargassum* dosage. Similarly, Isoleucine level in the control *Artemia* was 16.42mg/g and it increased from 17.81 to 19.36mg/g in Sargasssum fed Aremia. The maximum isoleucine level was observed at 750mg *Sargassum* dosage. Likewise, lysine content increased from 42.91mg/g to 45.28mg/g in *Sargassum* treated *Artemia* instead of 40.28 mg/g in the control. The maximum lysine content was noted at 750mg *Sargassum* dosage. As in the case mentioned above, methionine content increased from 10.51mg/g to 11.93mg/g in *Sargassum* treated *Artemia* instead of 9.21 mg/g in the control. The maximum methionine content was noted at 750mg *Sargassum* dosage. The phenylalanine level increased from 16.23mg/g to 17.92mg/g in *Sargassum* treated *Artemia* instead of 14.73 mg/g in the control. The maximum phenylalanine content was noted at 500mg *Sargassum* dosage. In the meantime, tryptophan level increased from 3.43mg/g to 3.97mg/g in *Sargassum* treated *Artemia* instead of 2.8 mg/g in the control. The maximum tryptophan content was noted at 500mg *Sargassum* dosage. Meanwhile, tyrosine level increased from 15.53mg/g to 16.70mg/g in *Sargassum* treated *Artemia* in place of 14.41 mg/g in the control. The maximum tyrosine content was noted at 500mg *Sargassum* dosage. The valine content increased from 14.61mg/g to 15.74mg/g in *Sargassum* treated *Artemia* instead of 12.40 mg/g in the control. The maximum tyrosine content was noted at 500mg *Sargassum* dosage. All these essential amino acids increased while increasing the *Sargassum* dosage from 250mg to 1000mg, but the maximum level was found at 500mg or 750mg/day. Even if the highest value was at 750mg dosage, the result was very close to the level observed at 5000mg dosage of *Sargassum* powder. The correlation coefficients of increase in the total essential amino acids were r= +23 ($P<0.05$) at 500mg dosage, r= +27 ($p>0.05$) at 500mg dosage, r= +26.2 ($p<0.05$) at 750 mg dosage and r= +26.1 ($p>0.005$) at 1000 mg dosage of *Sargassum* powder in relation to the values in the control.

Fatty acid Profile

There were significant differences in the fatty acid profiles of *Artemia* fed with rice bran and different gradations of *Sargassum* powder along with rice bran (Table-2). In the control, palmitic acid content was 1.67mg/g of *Aremia* and it increased from 1.85 to 1.93mg/g at 500mg *Sargassum* and then there was a steady state (750 and 1000mg doses). The peak of palmitic acid was noted at 500mg *Sargassum* powder. Stearic acid level in the control was 0.42mg/g and it increased

further from 0.67 to 0.83mg/g while increasing the dosages of *Sargassum* from 250mg to 500mg. The highest stearic acid level was noted at 500mg dose of *Sargassum* powder. Aracchidic acid level in the control Artemia was 0.13mg/g and it increased from 0.32 to 0.48mg/g in *Sargasssum* fed *Aremia*. The maximum aracchidic acid level was noted at 1000mg Sargassum dosage. Similarly, palmitoleic acid level in the control *Artemia* was 1.23mg/g and it increased from 1.41 to 1.52mg/g in *Sargasssum* fed *Aremia*; the highest amount was found at 750mg *Sargassum* dosage.

Likewise, oleic acid increased from 2.91mg/g to 3.04mg/g in *Sargassum* treated *Artemia* instead of 2.41 mg/g in the control; the highest amount was at 500mg *Sargassum* dosage. As in the case mentioned above, linoleic acid content increased from 0.51mg/g to 0.59mg/g in *Sargassum* treated *Artemia* instead of 0.49 mg/g in the control. The maximum linoleic acid content was noted at 500mg *Sargassum* dosage. The linoleinic acid level increased from 0.53mg/g to 0.71mg/g in *Sargassum* treated *Artemia* instead of 0.46 mg/g in the control. The maximum linolenic acid was noted at 1000mg *Sargassum* dosage. The EPA level increased from 0.94mg/g to 1.43mg/g in *Sargassum* treated *Artemia* instead of 0.86 mg/g in the control. The maximum tryptophan content was noted at 1000mg *Sargassum* dosage. Meanwhile, DHA content increased from 0.34mg/g to 0.49mg/g in *Sargassum* treated *Artemia* in place of 0.23 mg/g in the control; the maximum level was noted at 1000mg *Sargassum* dosage. Aracchidic acid, lioleic acid, EPA and DHA were found to be at maximum level at 1000mg dose of Sargassum while steraric acid, palmioleic acid, oleic acid and linoleic acid were in highest level at 500mg dose of *Sargassum* powder. The correlation coefficients of increase in the total fatty acids were r= +21 ($P<0.05$) at 500mg dosage, r= +26 ($p<0.05$) at 500mg dosage, r= +24 ($p>0.05$) at 750 mg dosage and r= +24.1 ($p>0.005$) at 1000 mg dosage of *Sargassum* powder per day.

DISCUSSION

Nutritional quality of *Artemia* is determined by its biochemical composition which varies primarily among the various geographical strains of *Artemia* as recorded by *Artemia* Reference Centre, Belgium (Beck and Bengston, 1982) and varies temporarily within a strain due to variation in the source and feed composition (Leger *et al*., 1986). According to Webb and Chu (1982), biochemical composition of *Artemia* also varies even in different batches of culture because of slight changes in growth conditions while Enright *et al.* (1986) had revealed that it is largely determined by nutrient content of the feeds used to culture *Artemia*. It was proved that nutritionally rich *Artemia* improves the growth rate, biomass and reproduction of aquaculture animals (Bengits *et al*, 1976; Van Ballaer *et al*., 1985; Gozalbo *et al*., 1987; Fernandez Retriz *et al*., 1991; Citarasu *et al*., 2002; Immanuel *et al*., 2004; Michael Babu *et al*., 2008). It is therefore necessary to produce sufficient quantity of nutritionally enriched *Artemia* to supply live feed to aquaculture industries (Tackaert, 1991). More like the other feeds being utilized in aquaculture, the *Artemia* also contains carbohydrates, proteins, lipids, free amino acids, free fatty acids, vitamins and minerals but its nutritional quality is mainly depending on the protein content (Kanzawa, 1991), lipids content (Schauer *et al*., 1980), amino acid profile (D'Abramo, 1991) and fatty acid profile (Bray *et al*., 1990). While all these components are in a balanced proportion in *Artemia*, the growth and reproductive performances of aquaculture animals are found to be in the maximum rate because of effective utilization of the feed components therein (Raj,1993), which may even be so critical for the growth of some species (Sorgeloos, 2001). It should be emphasized that total protein locked in the biomass of *Artemia* has been the inevitable nutrient for the growth and survival of aquaculture animals which are depending on *Artemia* as a live or dry feed (Seidal *et al*.,1980; Alava and Lin, 1983; and Tacon and Cowey, 1984). From the experiments of Ahmadi *et al.* (1990), Lee (1991) and Caric *et al.* (1993) it was obvious that the growth and survival of *Artemia* were high when there was 40-50 % protein in its body's dry weight. Nevertheless, the protein content of wild growing *Artemia* strains collected from various geographical regions of the world ranged from 52.90% to 74.04% (Gozalbo and Amat, 1988) and that in the Indian strains it ranged from 50 to 64% (Leger *et al*., 1986 and John 1994). Kanzawa (1991) had revealed that protein having an essential amino acid profile similar to that of aquaculture animal is best for the growth and survival of the animal in the culture system. The protein requirement of aquaculture animals was found to be 30-50% depending up on species, age and feeding habit (Millikin, 1982), so that even the wild growing *Artemia* can meet the protein requirement of aquaculture animals.However, the growth, survival and reproductive potentials of fishes were increased towards increase in the total protein content in *Artemia* feed (Gozalbo and Amat, 1998).

The protein content of Tuticorin strain of *A. parthenogenetica* was estimated to be 52.50 % which was further increased up to 56.4 % in cultures treated with the different doses of *Sargassum* powder. *Sargassum* itself contains about 12.3% crude protection, so that it might have increased the protein content of *Artemia* through enrichment (Gozalbo *et al*., 1987). Certain chemical constituents of *Sargassum* might have stimulated the biochemical pathways of *Artemia* concerned with feed utilization to increase the accumulation of some more amount of protein in its body. Protein content recorded in this study was equally good as recorded by John (1994) and Mony (1998). The results of the present study coincide with the findings of Royan (1980), Barghava *et al.* (1987), Gozalbo *et al.* (1987), Gozalbo and Amat (1988), Leger *et al.* (1986), John (1994) and Mony (1998). There are many convincing evidences to show that lipids in *Artemia* are important determiners of its nutritive value in the diet of aquaculture animals (Johns et al., 1980; Leger et al., 1984; Gozalbo *et al*., 1987; Citarasu *et al*., 2002; Tamaru et al., 2003; Immanuel *et al*., 2004; Michael Babu *et al*., 2008). Dietary lipids play crucial roles in energy metabolism (Michael Babu *et al*., 2008) as well as body weight gaining in the consumer organisms (Citarasu *et al*., 2002). The lipid content of wild growing *Artemia* strains collected from various geographical regions of the world ranged from 4.90% to 16.33 % (Gozalbo and Amat, 1988) and that in the Indian strains it ranged from 2-14% (Leger *et al*., 1986 and John (1994). Bray *et al.* (1990) had shown that fishes in aquaculture systems grow well while feeding them with *Artemia* containing 7.8-13.9% lipids in its body's dry weight but most of the wild *Artemia* could not meet the lipid requirement of the aquaculture animals. Several attempts have been made to increase the lipid content of *Artemia* by changing the feed compositions to boost up lipid production or by adding herbal extracts and Ayurvedic products doing a favour for it (Leger et al., 1984; Citarasu *et*

al., 2004; Michael Babu *et al*., 2008; Arularasu and Munuswamy, 2009). The lipid content of Tuticorin strain of *A. parthenogenetica* was estimated to be 13.40 % in the presence of rice bran, which was further increased up to 16.48 % in cultures treated with the *Sargassum* powder. The lipid concentration increased with increase in the concentration of dietary *Sargassum* powder, which implied that some amount of lipids might have passed to *Artemia* through enrichment process. Further, certain chemicals in *Sargassum* might have influenced the accumulation of lipids in the body of *Artemia*. Lipid level observed in this study was comparable to the lipid level recorded by John (1994) and Mony (1998). The results of the present study correlates with the findings of Royan (1980), Barghava *et al.* (1987), Gozalbo *et al.* (1987), Gozalbo and Amat (1988), Leger *et al.* (1986), John (1994), Mony (1998), Tamaru *et al.* (2003) and Ben Naucer *et al.* (2008). Amino acid profile of *Artemia* varies primarily with age of the cultures and secondarily with geographical strains (Seidal *et al*., 1980). Kanazawa (1991) suggested that protein whose amino acid profile is similar to that in a consumer organism is so ideal for the growth and survival of the organism. Castell *et al.* (1980) suggested that amino acid composition of *Artemia* varies depending on the feed composition and other growth conditions because of variations in nutrients supply. Amino acid profile of *Artemia* seems to influence the early larval growth, maturation, survival and reproduction in aquaculture animals (Ahmadi *et al*., 1990; and Claudia Aragoa *et al*., 2007).

Amino acid profile of *Artemia* grown in seawater medium fed with corn and soybean flour contained about 14.30mg (1.430%) of Asparagine, 17.26mg (1.726%) of Serine, 25.35mg (2.535%) of Glutamic acid, 21.02mg (2.102%) of Proline, 18.70mg (1.870%) of Glycine, 24.09mg (2.409%) of Alanine, 14.40mg (1.440%) of Valine, 10.60mg (1.060%) of Methionine, 16.68mg (1.668%) of Isoleucine, 22.08mg (2.208%) of Leucine, 3.60mg (0.360%) of Phenylalanine, 17.68mg (1.768%) of Histidine, 20.93mg (2.093%) of Lysine, 7.93mg (0.793%) of Cysteine, 3.90mg (0.390%) of Tryptophan and 28.46mg (2.846%) of Arginine per 1 gram of dried biomass (Schaver *et al*., 1980; AOAC, 1984 and Leger *et al*., 1986). Rice bran medium provide essential amino acids for the growth of *Artemia* while essential amino acids in the *Sargassum* power give an extra boost to accumulate still larger amount of essential amino acids in *Artemia*. In the present study, all the essential amino acids in the control *Artemia* were relatively less than those recorded in *Artemia* fed with different doses of *Sargassum* powder. Further, these amino acids increase with increase in the dosage of *Sargassum* powder up to 500mg dosage but further increase in the dosage does not favor the accumulation of essential amino acids. Accumulation of minerals in *Sargassum* might have interfered with the metabolism and subsequent storage of these amino acids in *Artemia* (Bhargava *et al*., 1987).

The proportions of Isoleucine, Cysteine and Leucine in this amino acid profile were relatively lower than those in the amino acid profile recorded by Leger *et al.* (1986) and AOAC (1984) while the concentrations of Proline, Alanine, Valine, Methionine, Phenylalanine, Histidine, Lysine, Tryptophan and Arginine were higher than those reported by Leger *et al.* (1986) and AOAC (1984). From these observations we can conclude that those feeds which influence the growth and survival have increased the accumulation of free amino acids in the *Artemia* according to their degree of growth stimulating activity. Regarding the amino acid profile of *Artemia* the results of the present study also coincide with the findings of Citarasu *et al.* (2002) and Immanuel *et al*. (2004). Free fatty acids in the diet are not only important for supplying calorific energy but also for providing essential polyunsaturated fatty acids (PUFA) required for normal cellular functions in aquaculture animals (Esteveze and Kanazawa, 1996). Among the PUFAs, the Eicosapentaenoic acid [C20:5 n-3] (EPA) is more potent than Docosahexaenoic acid [C22:6n-3] (DHA) in influencing the growth and survival of marine fishes and other aquaculture animals; both EPA and DHA are utilized in the synthesis of polyunsaturated long chain fatty acids (Shields, 1999). According to Leger *et al.* (1986), the saturated fatty acids like Palmitic acid (C16:0) and Arachidic acid (C20:0) and the unsaturated fatty acids like Oleic acid (C18:1), Linoleic acid (C18:2), Alpha linoleic acid (C18:2) and Stearic acid (C18:2) are very important in the diet of fishes for enhancing the larval growth. *Artemia* biomass having high proportion of these fatty acids is therefore more likely in the diet of aquaculture animals.

Stanley-Samuelson (1994) has shown that long chain n-3 fatty acids are required for the early nervous system development in fishes and shrimp while n-6 fatty acids such as arachidonic acid is a precursor of prostaglandins and some other biologically active compounds which regulate the growth and reproductive functions in aquaculture animals. By the way of changing the nutritional composition of feeds or by adding some inert components that do not serve as feed substance but enable *Artemia* to synthesize more fatty acids, the fatty acid profile of *Artemia* can be manipulated so as to enrich it with essential fatty acids and other long chain fatty acids which are required to meet the fatty acid requirement of aquaculture animal for enhancing their survival, growth and reproductive performance in the culture systems (Triantaphyllidis et al., 1993; Sargent et al., 1995; Esteveze and Kanazawa, 1996; Evjemo et al., 1997; Kyungmin, 2000; and Ben Naceur *et al*., 2003). It was also proved that the fatty acid profile of *Artemia* also varies depending up on the geographic strains so that strain selection will also be very useful to produce nutritionally rich *Artemia* biomass (Vanhaecke and Sorgeloos, 1980; Bell *et al*., 1995; Bengtson *et al*., 1991; Kovan *et al*., 2000 and Kara *et al*., 2004). According to Tamaru *et al.* (2003), *Artemia* takes up the fatty acids from dietary components and its fatty acid profile changes according to the duration of the enrichment period. Newly hatched *Artemia* had 7.0 mg total fatty acids/100 mg dry weight and had no detectable levels of DHA (docosahexaenoic acid). After enrichment for 12 h, the total fatty acids increased significantly to 10.3 mg/100 mg and the *Artemia* had significantly higher amounts of essential fatty acids in the n-3 and n-6 families. After 24 h of enrichment, significantly higher levels of essential fatty acids and total fatty acids were achieved.

Their study indicated that the duration of the enrichment process should be considered when preparing *Artemia* as a food for larvae of the ornamental fish. In the present work, *Sargassum* was used as a supplementary diet to boost up the fatty acids and essential amino acids in *Artemia* for enhancing their nutritive value to promote the fast growth of aquatic animals. This enrichment procedure can be used to increase the fatty acid composition of *Artemia* so as to make it an ideal

feed to aquaculture fishes (Coutteau *et al*., 1997; Evjemo *et al*., 1997; Kyungmin, 2000; Van Stappen *et al*., 2003; Ben Naceur *et al*., 2008 and Michael Babu *et al.* 2008). The resultant fatty acid profiles of *A. parthenogenetica* are equally as good as those reported by Leger *et al.* (1986), AOAC (1984), Evjemo *et al*.(1997), Reiss *et al.* (1998), Kyungmin (2000), Van Stappen *et al.* (2002) and Ben Naceur *et al.* (2008). It is therefore concluded that 500mg *Sargassum* powder may be added to *Artemia* cultures for enhancing its nutritive value for using it as dietary supplement to fishes, animals and humans.

The fatty acid profile of *Artemia* grown in seawater medium fed with corn and soybean flour contained about 9.10mg (0.910%) of Palmitic acid, 2.81mg (0.281%) of Stearic acid, 13.20mg (1.320 %) of Oleic acid, 2.02mg (0.2102%) of Linoleic acid, 0.074mg (0.0074%) of Alpha linoleic acid, <0.5mg (0.05%) of Aracchidic acid, 0.03mg (0.003%) of EPA and 1.210mg (0.121%) of DHA per 1 gram of dried biomass (Leger et al., 1986). In the present study, there was a rise in the proportion of almost all these individual fatty acids in the *Artemia* grown in the presence of *Sargassum* powder than that in the control. Further, the concentrations of these fatty acids were little higher than those recorded by AOAC (1984) and Leger *et al.* (1986), which might be due to stimulation of metabolic pathways that convert the sugars into fatty acids by the action of chemical components in the *Sargassum* powder. The concentration of EPA and DHA was higher in *Artemia* grown in rice bran with Sargassum powder than *Artemia* grown in corn flour and soybean powder. However, the accumulation of Aracchidic acid was relatively lower than that in *Artemia* grown on corn and soybean flour because rice bran is deficient in Aracchidic acid. As far as the composition of various fatty acids in the fatty acid profile of *Artemia* is concerned, the results of this investigation agrees with fatty acid profiles discovered by Sastry (1985), Leger *et al.* (1986), Takeuchi *et al.* (1994), Sargent *et al.* (1995) and Esteveze and Kanazawa (1996).

Conclusion

The present investigation concludes that *Sargassum wightii* powder is a suitable feed supplement to *Artemia* for enhancing the total proteins, lipids, essential amino acids and fatty acids required for growth and reproductive capabilities of aquatic animals. Since *Sargassum* powder contains almost all components found in rice bran, it can be substituted for rice bran in the culture of *Artemia*. This feed supplement enables the *Artemia* to accumulate more amounts of proteins, lipids, essential amino acids and fatty acids, which make it a suitable live feed for aquaculture animals. Nutritional enhancement effect is the maximum at 500mg dosage of *Sargassum* but higher dosages (750 and 1000mg) do not show marked impact since *Artemia* cannot intake more feed supplements. If a stress is created in the *Artemia* culture, the vigorously growing *Artemia* can produce a large amount of cysts in the culture, which is more likely in the commercial hatcheries.

Conflict of interest

The author declares that she has no conflict of interest.

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