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Effect of alginate supplemented feed on biochemical composition of white shrimp *Litopenaeus vannamei* (Crustacean/Penaeid)

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Abstract
Marine crustaceans are important source of food in many countries and are known for their economic value. The present study was carried out to assess the biochemical composition of *Litopenaeus vannamei* cultured under different percentage of alginate supplemental diet. The results showed that protein, carbohydrate and lipid varied significantly between different alginate supplemented diet groups of *L. vannamei* on 15th and 30th day ($P < 0.05$). Essential amino acids were represented by arginine, cysteine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, tyrosine and valine and non- essential amino acids were alanine, asparagine, aspartic acid, glycine, proline, glutamine and serine. Compared to control, fatty acids (SFA, MUFA and PUFA) and minerals (Ca, Mg, Zn, Fe and P) showed higher content in the tissues of *L. vannamei* fed with diet supplemented with alginate. Our study showed that the shrimps fed with diet supplemented with alginate had a positive effect on biochemical composition.

Keywords: *Litopenaeus vannamei*, Alginate, Amino acids, Fatty acids, Minerals, biochemical composition

1. Introduction
Edible marine crustaceans are the major source of nutritious food for human beings. The nutritive value depends upon their biochemical composition, such as protein, amino acids, lipids, fatty acids, carbohydrates, vitamins and minerals [1]. Due to their nutritious nature, they play an important role in maintenance of physiological and biochemical activities in human beings. *Penaeus monodon*, *P. indicus*, *P. semisulcatus*, *Metapenaeus monoceros* and *M. dobsoni* are considered to be important commercial species available in India [2]. Among the above species, *Litopenaeus vannamei* are considered to be most prominent promising candidate species for culture activity in India [3]. So far, the focus has been to evaluate diet quality in terms of growth parameters of the cultured species, but its effect on biochemical composition is less. Therefore, the present study is aimed to probe into effective use of sodium alginate as feed supplement and its effect on biochemical composition, amino acids, fatty acids and mineral content in the muscle of *L. vannamei.*

2. Materials and methods
2.1 Preparation of test diets
Sodium alginate was extracted from *Turbinaria decurrens* by the method described by Suzuki (1955) [4] and Ganesan *et al.*, (2001) [5]. The extract was mixed with commercially available artificial (Taiwan) pellet feed. The three types of alginate supplemental feed prepared were Type (1)-standard (sodium alginate-Sigma product); Type (2)-1% and Type (3)-2 % was prepared using the extract and control (without alginate). The feed was given to shrimp according to the method followed by Balasubramanian *et al.*, (2008) [6].

2.2 Collection and maintenance of experimental shrimp
Litopenaeus vannamai (n=150) were collected from culture pond near Sirkali (Lat. 11°29" N and Long. 79°46" E), Tamil Nadu, South East coast of India. Shrimps were cultured in circular tank (150 L) and acclimated to the laboratory conditions for 15 days before the experiment. During acclimation period, the shrimps were fed twice (7.30 am and 5.30 pm) daily with artificial pellet feed. Aeration was provided at regular interval and 50% of water was exchanged daily to maintain the water quality in the tanks. During the culture periods, water temperature, 29.0±0.5°C; pH, 7.5±1.3 and salinity 32±3.1% was maintained. Shrimp length ranged from 7.4 to 9.2 cm and weight 8.1 to 9.6 g was used for the experiment.

2.3 Determination of Proximate Composition
The estimation of moisture content was carried out by hot- air oven method, protein by the method of Lowry *et al*. (1951) [7], carbohydrate by Dubois *et al*. (1956) [8], lipid content by Folch *et al*. (1957) [9] and minerals analyzed by AOAC, (1990) [10].
Amino acid was determined using Lawrance Evans, (2007) [11] method. The samples were quantified using HPLC column. The test solution was concentrated to 1.0 mg/ml with a reference solution of mixed amino acids on cold rolled steel as the mobile phase of concentration 1.0 mg/ml. Separation of amino acids was achieved using octadecylsilyl silica capillary column (size: l=0.10 m, Ø = 4.6 mm) and the mobile phase, trimethylamine. The flow rate was 1.0-1.5 ml/min (run time: 90 min). 20 µl of test solution was added and read using spectrophotometer at 220 nm. The results were expressed as % of amino acid with respect to total amino acids. Fatty acid content was determined using the Hong Wang, (2007) [12] method. Fatty acids were analyzed using

gas chromatography equipped with a flame ionization detector. The separation was achieved using a fused silica capillary column (30 m × 0.25 mm × 0.25 μm film thickness). The oven temperature was set at 1700C for 55 min. The injector and detector temperatures were maintained at 2500C and 2800C, respectively. The carrier gas was hydrogen or helium for chromatography with a spit ratio of 1/200. The fatty acids were determined using differential refractometer. The columns contained styrene-divinylbenzene copolymer column (0.3 m × 7.8 mm × 7 μm film thickness).

2.4 Statistical analysis

To know the statistical significance one way ANOVA was carried out for the biochemical composition between different diets of *L. vannamei* on 15th day and 30th day.

3. Results and Discussion

The biochemical composition of *L. vannamei* fed with different diets on 15th and 30th day is given in the Table 1 & 2. In the present study the protein content varied from 17.62±0.72 to 25.57±0.2 %, carbohydrate varied from 2.21±0.54 to 4.97±0.3 %, lipid from 1.15±0.1 to 3.67±0.7 %, ash content varied from 3.94±0.12 to 6.0±0.16 % and moisture from 70.03 ±0.02 to 71.02± 0.02 % on 15th day. On the 30th day the protein content fluctuated from 23.79±0.3 to 31.22±0.5 %, carbohydrate from 2.44±0.4 to 6.45±0.11 %, lipid from 1.24±0.8 to 4.56±0.36%, ash content from 5.42±0.16 to 5.60±0.21 % and moisture content from 71.05±0.02 to 73.34±0.02 % respectively on 30th day.

Earlier reports showed significant effect of different types and levels of carbohydrates in the diet. It was indicated that *P. monodon* utilize trehalose and sucrose better than glucose[13]. The same condition has also been reported for other penaeid species such as *P. japonicus* [14, 15, 16], *P. setiferus* [17] and *P. duorarum* [18]. But the mechanism of the poor consumption of glucose is not fully understood so far [19]. In the present study, alginate - a seaweed polysaccharide abundant in the cell wall of brown algae is used as a feed supplement. Already alginate had been used as immunostimulant in aquaculture to improve the health status of animals [20, 21, 22]. But its effect on biochemical composition of aquatic animals is limited. Hence, in the present study an attempt was made to study the effect of alginate on biochemical composition of *Litopenaeus vannamei*.

The results of one way analysis of variance showed that ash and moisture did not varied significantly between different diets groups of *L. vannamei* whereas protein, carbohydrate and lipid varied significantly between different alginate supplemented diet groups of *L. vannamei* on 15th and 30th day ($P < 0.05$) (Table 3&4). The differences observed in the proximate composition of shrimp flesh could be due to the supplementation of alginate. The results clearly reveal the growth promoting potential of alginate diet in *L. vannamei.* Similar results were reported on the growth and survival of larvae of *P. japonicus* were feeding trials using purified diet with carrageenan as a binder was used [23].

Amino acid composition consisting of essential (EAA) and non-essential amino acid (NEAA) of *L. vannamei* is given in Table.5. *L. vannamei* had all the essential amino acids that are required for the normal functioning of the body. Though the ratio of EAA to NEAA in different diet groups of *L. vannamei* showed more or less same values, but it is higher than *P. monodon* [24, 25] and *P. semisulcatus* [26]. The ratios is 1.05 and 1.09 in prawns and periwinkles respectively. Thirteen amino acids were reported, 8 essential amino acids and 5 non-essential amino acids. Glutamine, asparagine, lysine, leucine, arginine, glycine and valine were the most abundant amino acids. Generally, it is reported that the amino acid content varies by intrinsic factors (species, size and sexual maturity) and extrinsic factors (food resources, fishing season, water salinity and temperature) so in the present study the variations may be due to alginate supplemented feed [27, 28].

Variations in the level of fatty acid were found in different diet groups. The level of order for saturated fatty acid was found to be standard >2%>1%>control, for mono unsaturated fatty acid it was standard>1%>2%>control and polyunsaturated fatty acid was 1%>standard>2% control respectively. A crustacean lipid is affected by changes in environmental factors. Linoleic acid and n-6 polyunsaturated fatty acids are predominant fatty acids in freshwater shrimp whereas linolenic acid and n-3 highly unsaturated fatty acids predominate in marine crustaceans. Similar results are reported in present study (Table.6).

The mineral content in *L. vannamei* is shown in (Table.7). Changes in the level of Ca (mg/g dry wt) in *L. vannamei* tissues appeared in the following order: 2 % >standard > 1 % > control; and for Mg and Zn (mg/g dry wt) the level appeared as 2 %> 1%>standard>control. Likewise, the level of Fe and P (mg/g dry wt) followed order as 1% >standard>2%>control. Based on the results i.e. high mineral content in the tissues of *L. vannamei* fed with alginate supplemented diet than the control it can be concluded that alginate had a positive effect on biochemical composition. Shrimp absorb most of the minerals from the water by direct absorption via the gills, skin or both [29]. At the same time alginate is also well known for absorption of elements directly from the surrounding waters so may be this would have enhanced the absorption of minerals into the tissues of *L. vannamei.*

Table 1: Effect of different diets on biochemical composition of *L. vannamei* on 15th day

Parameters	Protein (%)	Carbohydrates (%)	Lipid (%)	Ash (%)	Moisture (%)
Control	17.62 ± 0.72	2.21 ± 0.54	1.15 ± 0.1	6.0 ± 0.16	71.02 ± 0.02
Standard	22.57 ± 0.2	4.06 ± 0.29	3.67 ± 0.7	4.93 ± 0.11	70.03 ± 0.02
1%	21.68 ± 0.1	3.95 ± 0.14	1.85 ± 0.35	3.94 ± 0.12	70.05 ± 0.02
2%	25.57 ± 0.2	4.97 ± 0.3	3.05 ± 0.15	5.0 ± 0.19	70.06 ± 0.02

Table 2: Effect of different diets on biochemical composition of *L. vannamei* on 30th day

Parameters	Protein (%)	Carbohydrates (%)	Lipids (%)	Ash (%)	Moisture (%)
Control	23.79 ± 0.3	2.44 ± 0.4	1.24 ± 0.8	5.60 ± 0.21	73.34 ± 0.02
Standard	28.55 ± 0.4	5.03 ± 0.12	4.56 ± 0.36	5.52 ± 0.12	72.46 ± 0.02
1%	27.70 ± 0.34	4.91 ± 0.41	2.98 ± 0.2	5.55 ± 0.13	72.36 ± 0.02
2%	31.22 ± 0.5	6.45 ± 0.11	4.26 ± 0.21	5.42 ± 0.16	71.05 ± 0.02

Table 3: One way ANOVA for the biochemical composition between different diets *L. vannamei* on 15th day

		SS	*df*	*MS*	*F*	Sig.
Protein	Between Groups	129.332	3	43.11065	5.830	P<.05
	Within Groups	88.72375	12	7.393646		
	Total	218.0557	15			
Carbohydrate	Between Groups	15.95357	3	5.317856	3.657	P<.05
	Within Groups	17.44768	12	1.453973		
	Total	33.40124	15			
Lipid	Between Groups	15.51057	3	5.17019	4.837	P<.05
	Within Groups	12.82468	12	1.068723		
	Total	28.33524	15			
Ash	Between Groups	8.4971	3	2.832367	0.556	NS
	Within Groups	61.0872	12	5.0906		
	Total	69.5843	15			
Moisture	Between Groups	2.844	3	0.948	1.001	NS
	Within Groups	11.3616	12	0.9468		
	Total	14.2056	15			

Table 4: One way ANOVA for the biochemical composition between different diets *L. vannamei* on 30th day

		SS	*df*	*MS*	*F*	*Sig.*
Protein	Between Groups	113.5946	3	37.86485	5.240	P<.05
	Within Groups	86.70595	12	7.225496		
	Total	200.3005	15			
Carbohydrate	Between Groups	33.2915	3	11.09717	4.850	P<.05
	Within Groups	27.4564	12	2.288033		
	Total	60.7479	15			
Lipid	Between Groups	27.43528	3	9.145092	4.055	P<.05
	Within Groups	27.0571	12	2.254758		
	Total	54.49238	15			
Ash	Between Groups	0.069669	3	0.023223	0.521	NS
	Within Groups	0.534175	12	0.044515		
	Total	0.603844	15			
Moisture	Between Groups	10.6931	3	3.564367	2.015	NS
	Within Groups	21.2184	12	1.7682		
	Total	31.9115	15			

Table 5: Amino acid composition (%) in shrimps on 30th day

Amino acid composition (%)				
Essential Amino Acids (EAA)	**Control**	**Standard**	**1%**	**2%**
Arginine	1.32	1.44	1.33	1.58
Cysteine	1.41	1.38	1.41	1.66
Histidine	0.79	1.34	1.03	1.26
Isoleucine	3.26	1.21	1.39	1.38
Leucine	0.76	1.44	2.09	1.26
Lysine	1.4	1.69	1.77	0.68
Methionine	0.96	0.75	1.03	0.62
Phenylalanine	0.97	1.38	1.48	1.57
Threonine	1.55	1.03	1.07	1.06
Tryptophan	0.6	0.42	0.33	0.18
Tyrosine	0.98	1.4	1.1	1.55
Valine	1.05	0.43	0.5	0.59
Non-essential amino acids (NEAA)				
Alanine	2.26	2.01	2.1	2.61
Asparagine	3.04	2.58	2.56	2.89

Aspartic acid	2.39	2.42	2.45	2.81
Glycine	1.8	1.39	1.49	1.38
Proline	0.75	0.5	0.68	0.94
Glutamine	1.39	1.05	1.01	1.01
Serine	1.73	2.72	2.69	2.26
EAA/ NEAA	1.12	1.09	1.11	0.96

Table 6: Fatty acid composition (%) in shrimps on 30th day

Fatty acids	Control	Standard	1%	2%
Saturated fatty acids (SFA)				
Palmitic acid (C16:0)	17.96	16.14	18.7	25.87
Margaric acid (C17:0)	11.07	8.28	8.76	8.67
Stearic acid (C18:0)	1.57	23.22	12.6	6.13
Mono unsaturated fatty acid (MUFA) (C18:1n-9)				
Oleic acid	10.48	21.11	20.86	16.92
Poly unsaturated fatty acid (PUFA)				
Linolenic acid (C18:2n-6)	14.37	19.3	20.57	20.11
Alpha Linolenic acid (C18:3n-3)	9.84	10.7	11.75	7.17
Morotic acid (C18:4)	2.51	5.33	4.37	6.2
PUFA/SFA	0.87	0.74	0.91	0.82
MUFA/SFA	0.34	0.44	0.52	0.17
Saturated fatty acids ΣSFA	30.6	47.64	40.06	40.67
Mono unsaturated fatty acid ΣMUFA	10.48	21.11	20.86	16.92
Poly unsaturated fatty acid ΣPUFA	26.72	35.33	36.69	33.48

Table 7: Minerals content (mg/g dry wt) in shrimps on 30th day

Parameters	Calcium	Magnesium	Zinc	Iron	Phosphorus
Control	256.7	102.6	9.34	7.55	65.6
Standard	356.7	122.6	15.6	10.45	134.5
1%	332.6	134.7	22.9	11.6	145.7
2%	444.7	256.7	35.6	9.45	89.4

4. Conclusion

Shrimp continued to be a high economic cultivable species due to its high market price and demand. Many problems remain to be solved regarding the development of marketable size species under high density. In this regard, artificial feeds play an important role in every phase of shrimp culture starting from larval rearing to broodstock maturation and spawning. The present study showed that the shrimps fed with diet supplemented with alginate had a positive effect on biochemical composition. So, further studies are needed for successful development of nutritionally complete and cost-effective diets.

5. Acknowledgments

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6. References

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