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Influence of dietary supplementation of sodium alginate on gut flora and biochemical composition in *Fenneropenaeus indicus*(Indian major shrimp)

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ABSTRACT

In the present investigation, the influence of dietary supplementation of sodium alginate (extracted from Turbinaria decurrens) on the gut flora of the Fenneropenaeus indicus(Indian major shrimp)was studied. Shrimps were cultured in the laboratory for 30 days and various concentration of sodium alginate along with commercial diet was tested. The total heterotrophic bacterial load ranged from 4.4 × 10^2 CFU/g to 11.2×10^2CFU/g of gut sample. The maximum number of colonies were found in the gut of shrimp fed with Type 4 diet (1% sodium alginate) and minimum in control. The dominant strains isolated were identified as Pseudomonas aeruginosa, Bacillus licheniformis, Bacillus badius and Vibrio parahaemolyticus. The protein, carbohydrate and lipid values were significantly different between the different concentrations of sodium alginate diet (P < 0.05).The water quality variables that most influenced the tank water were the total ammonia nitrogen (TAN), dissolved oxygen (DO), dissolved inorganic phosphorus (DIP) and silicate (Si). The present study clearly showed that diets containing sodium alginate influence the numbers of beneficial gut microflora and biochemical composition in shrimps.

Keywords: Sodium alginate, gut microbes, biochemical composition, water analysis, *Fenneropenaeus indicus*

INTRODUCTION

The aquaculture industry is growing tremendously. Pathogenic microorganisms have a critical role in the aquaculture field because the water quality and disease control are directly related and closely affected by the microbial activities[1]. Gut flora has a continuous and dynamic effect on the host's gut and systemic immune systems. The important role of gut microbes in each host animal is in digestive process, growth and disease [2].

The ability of the gut flora to degrade algal polysaccharides and other complex plant polymers in an animal's diet can increase its host's digestive efficiency. The major polymers of the ingested epiphytic algae include starch, found in the green algae, and cellulose in the green, brown and red algae [3]. In addition to gut microbiota, excluding the adhesion of other species to the intestinal wall, the bacteria can also out-compete pathogens for carbon and energy sources in the aquatic environment [4].

Best management practices are required both in hatchery and pond system to obtain good results. Nowadays, researchers are trying to use bacteria in aquaculture to improve water quality by balancing bacterial population in water and reducing pathogenic bacterial load. Careful control of water temperature, salinity, pH, optimization of stocking densities and balanced nutrition are also very important to improve survival [5]. It has an additional beneficial effect on the host by modifying the host associated microbial community, ensuring improved use of the feed and its nutritional value. Also, it is enhancing the host response towards disease, thereby improving the quality

of its ambient environment. The potential application of useful bacteria to aquaculture feeds, especially in fish and shellfish larviculture, has been highly investigated [6]. However, the shrimp industry suffers from repeated appearance of diseases that affect the sustainability of aquaculture [7]. Now, one of the methods that is gaining recognition for controlling pathogens within the aquaculture industry is the use of beneficial microorganisms [8].The aim of the present study was to isolate and identify the bacterial gut flora, and to analyze the diversity of bacteria based on different sodium alginate diet.

MATERIALS AND METHODS

Preparation of test diets

The sodium alginate extracted from *Turbinaria decurrens*[9, 10] was used for the present experimental work. The extract was mixed with commercially available pellet feed. The four types of formulated dietwasType (1)- control (without alginate); Type (2)- standard (sodium alginate- Sigma product); Type (3) - 0.5 %; Type (4) - 1 % and Type (5)- 2 %) was prepared using the extract. For coating, the feed pellets was mixed with the extract solution and incubated at room temperature for 7 days. After the absorption of the extract, it was coated with the binding gel to prevent dispersion of the extract in water. The feed was then dried and stored at room temperature until given to shrimp [11].

Collection and maintenance of experimental shrimp

Fenneropenaeus indicus (Indian major shrimp) were collected from Vellar estuary (Lat. 11º29″ N and Long. 79º46″ E), Tamil Nadu, South East Coast of India. Shrimp length ranged from 7.3 to 9.0 cm and weight 8 to 9.5 g. Shrimps (n=150) 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 commercial pellet feed. Aeration was provided at regular intervals and 50 % of water was exchanged daily to maintain the water quality.

Isolation of the gut and homogenization

The samples were collected on 1st and 30th day from different tanks. Shrimps were anaesthetized in an ice bath for 5–10 min and each individual surface sterilized by immersion for 30 seconds in 70 % ethanol. The gut was aseptically dissected from the animal's musculature. Gut was placed into a 10 ml sterile double strength phosphate-buffered saline solution, 0-6 % sodium phosphate and 1-2 % sodium chloride and homogenized in tissue homogenizer.

Isolation and identification of bacteria

One ml aliquot of the gut homogenate was aseptically spread with 9 ml sterile double strength phosphate-buffered saline onto nutrient agar. All plates were incubated at 37℃ for 24-48 hrs. The numbers of cultivable bacterial cells present in shrimp gut were estimated after isolation and growth on nutrient agar plates incubated at room temperature. Colonies developed on the plate were counted and expressed as CFU/g. For the identification, the isolates obtained were purified on the agar slants. The isolated bacterial strains were identified up to species level. A series of secondary tests was also performed to complete the genus level identification of isolates [12].

Analysis of proximate composition

The protein, carbohydrate and lipid contents were estimated in the shrimps collected on 1st and 30th day by adopting the standard methods [13-15]. The results were expressed in percentage.

Hydrological sampling and chemical analysis

Water temperature (T, ˚C), salinity (%) and pH were monitored at 0600 h and 1800 h.To characterize the water quality adequately, culture tanks were sampled on 1st and 30th day. The water samples were collected using polyethylene plastic bottles and kept at 4 °C until analysis. The water quality variable studied were viz., dissolved oxygen (DO), total ammonia nitrogen (TAN), nitrate nitrogen (NO_3-N), nitrite-nitrogen (NO_2-N), dissolved inorganic phosphorus (DIP) and silicate (Si).

Statistical analysis

Correlation structure between the variables was studied using the Spearman R coefficient as a non-parametric measure of the correlation between the variables. All the statistical computations were made using Microsoft Office Excel 2007 and SPSS 11.0.

RESULTS AND DISCUSSION

The composition of the bacterial community in an aquaculture environment has a strong influence on the internal bacterial flora of cultured animals, which is vital for their nutrition, immunity and disease resistance [16].The gut micro biota of fish had been studied extensively using various techniques. The isolates were identified by various biochemical tests. *Pseudomonas*, *vibrio* and *Bacilli* are the common bacteria found in *Oreochromis mossambicus*, *Oreochromis leucostictus* and *Etroplus suratenis*[17]. Similarly, *Bacillus* was isolated from channel catfish [18] goldfish [19] and Atlantic cod [20].

Table 1. Biochemical characteristics of the bacterial isolates

S. No.	Biochemical test	Stain 1	Stain 2	Stain 3	Stain 4
1.	Growth on NA (24 hrs)	Flat colonies, 3mm With bluish green diffusible pigment	Dry flat spreading opaque colonies, 2mm	Dry flat Spreading opaque colonies, 2-3mm	green colour
2.	Gram staining	Gram negative rods	Gram positive rods	Gram positive rods	Gram negative rods
3.	Catalase	positive	positive	positive	positive
4.	Oxidase	positive	negative	negative	positive
5.	Motility	motile	motile	motile	motile
6.	Nitrate	positive	positive	negative	
7.	Indole	negative	negative	Negative	positive
8.	Methyl red test	negative			
9.	Voges-proskauer test	negative			negative
10.	Citrate	positive			positive
11.	H_2S	negative			
12.	Growth in mac	Positive			
13.	Urease	negative			
14.	Arginine dihydrolase	positive	Parabasal crystals negative	Parabasal crystals negative	positive
15.	Esculin	Negative		Parasporal bodies negative	
16.	Starch hydrolysis	negative	positive		
17.	Gelatin hydrolysis	positive		positive	
18.	Fermentation				
	Glucose	positive			positive
19.	Lactose	negative			negative
20.	Sucrose	negative			negative
21.	Mannitol	positive			positive
22.	Xylose	positive			
23.	Trehal		positive	negative	
	Species	*Pseudomonas aeruginosa*	*Bacillus licheniformis*	*Bacillus badius*	*Vibrio parahaemolyticus*

Earlier investigation of gut flora of fresh water fish (*Garramullya*) (Sykes) revealed bacterial strains showing wide diversity of enzyme production, morphological and biochemical characteristics. The factors which govern the diversity showed that the strains have adaptation to exploit variety of resources like citrate and sugar, and also shown to tolerate variety of environmental conditions like pH, salt, and bile in *in-vitro* conditions. These reasons shows that the study of bacterial flora of the aquatic animal gut is important. In general, microbiota provide metabolic, trophic and protective functions in marine organisms. There are only few reports on the gut flora of shrimps. In the present study, the total heterotrophic bacterial load ranged from 4.4×10^2CFU/g to 11.2×10^2 CFU/g of gut sample.

The role of gut microbes as probiotics is reported by many workers [21, 22]. *Pseudomonas* sp. was used to induce resistance to bacterial infections and improve survival in larval cultivation of *Argopecten purpuratus*. *Bacillus* possess adhesion abilities, produces antimicrobial peptides and provide immunostimulation [23-25]. It was also demonstrated that *Bacillus* isolates are good choice in larviculture to improve health, rate of development, and rate of survival of white leg shrimp [26].In the present study, the maximum number of colonies were found in the gut of shrimp fed with 1% sodium alginate diet and minimum in the control diet. The dominant strains isolated were identified as *Pseudomonas aeruginosa*, *Bacillus licheniformis*, *Bacillus badius*, and *Vibrio parahaemolyticus* Table.1.

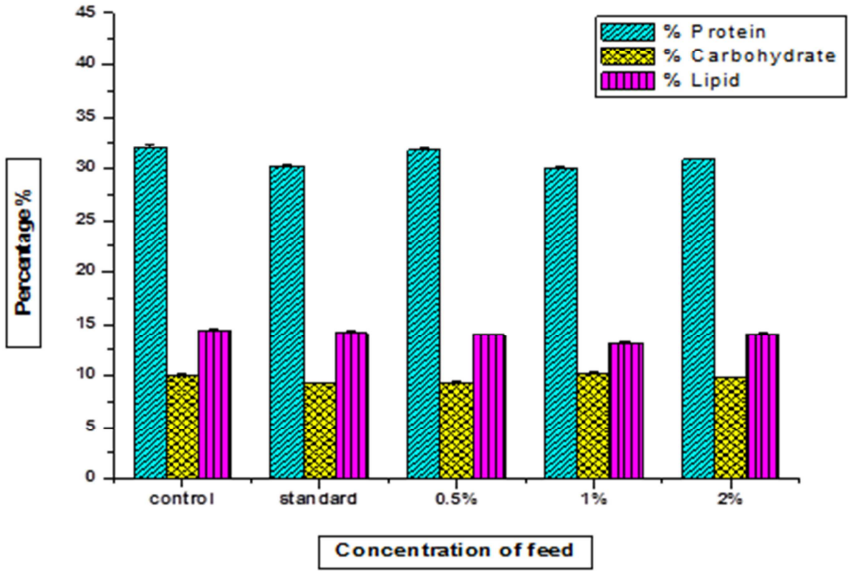

Fig .1. Biochemical composition of shrimp fed with different diets on 1st day

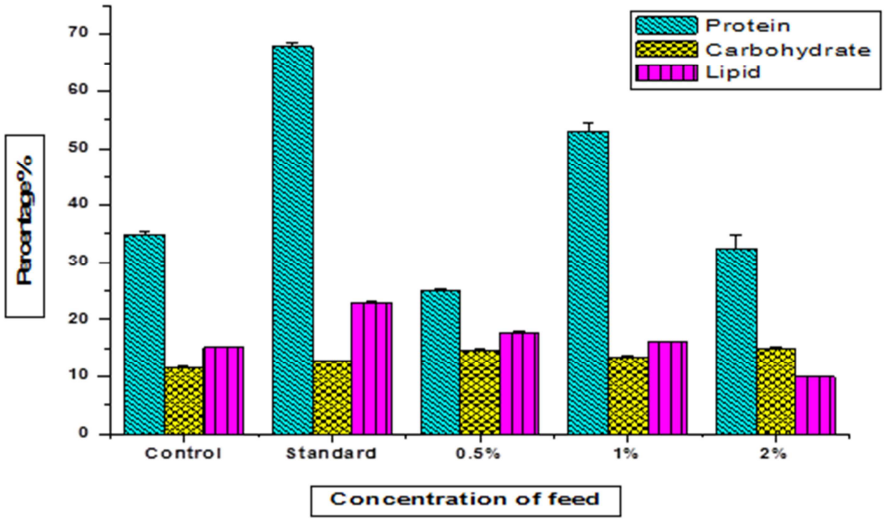

Fig. 2. Biochemical composition of shrimp fed with different diets on 30th day

In the earlier reports *Aeromonas, Plesiomonas, Photobacterium, Pseudomonas, Pseudoalteromonas* and *Vibrio* were identified from different parts of the *Penaeus merguiensis* digestive tract [27].Higher bacterial population in the stomach contents has been observed in prawns, feeding upon the epiflora, epifauna and the organic matter rich in bacteria from mud substrates. The present study also shows that diet supplemented with sodium alginate enhances the numbers of beneficial gut flora than in the control diet.

The proximate composition of tissues of *Fenneropenaeus indicus* are shown in Fig 1 & 2. The protein, carbohydrate and lipid values were significantly different between the different concentrations of sodium alginate diet ($P < 0.05$). On the 1st day the protein, carbohydrate and lipids content ranged from 30.04 to 32.05 %, 9.24 to10.15 % and 13.5 to 14.5 % respectively, for the shrimps collected from different tanks. On the 30th day higher value for protein was found in shrimps fed with Type 2 diet (68.03 %) and in Type 4 (54.01 %), whereas it was lower in Type 1 diet

(control). Carbohydrate content was high in Type 5 (15.50 %) and in Type 3 (15.0 %). At the same time lipid content was high in Type 2 (24.1 %) and in Type 3 (18.01%). Minimum values were recorded for Type 5 (10.02 %), when compared to control. The present study clearly shows that the diet had a significant effect on the proximate composition of muscle tissues of *Fenneropenaeus indicus*. Similar effect of supplemented diet using herbal medicinal plants was reported in *Penaeus monodon*[28] and in *Fenneropenaeus indicus*[29].

Correlation with water quality variables

Correlation structure between the variables was studied using the Spearman R coefficient as a non-parametric measure of the correlation between the variables (Table 2,3,4,5 & 6).

Table 2. Correlation between the different water parameter using the Spearman R coefficient - control tank

	DO	TAN	NO_3-N	NO_2-N	DIP	Si
DO	1					
TAN	.801	1				
NO_3-N	.532	-.082	1			
NO_2-N	-.983	-.897	-.368	1		
DIP	.898	.983	.105	-.963	1	
Si	.327	-.304	.974	-.149	-.122	1

** correlation is significant at the 0.05 level (1-tailed)*
*** correlation is significant at the 0.01 level (1-tailed)*

Table 3. Correlation between the different water parameter using the Spearman R coefficient - standard sodium alginate (Sigma product)

	DO	TAN	NO_3-N	NO_2-N	DIP	Si
DO	1					
TAN	.998*	1				
NO_3-N	-.425	-.485	1			
NO_2-N	-.674	-.623	-.382	1		
DIP	-.891	-.858	-.034	.936	1	
Si	.992*	.998*	-.537	-.574	-.825	1

** correlation is significant at the 0.05 level (1-tailed)*
*** correlation is significant at the 0.01 level (1-tailed)*

Table 4. Correlation between the different water parameter using the Spearman R coefficient - 0.5 % of sodium alginate (Extract)

	DO	TAN	NO_3-N	NO_2-N	DIP	Si
DO	1					
TAN	.993*	1				
NO_3-N	-.318	-.431	1			
NO_2-N	-.891	-.829	-.147	1		
DIP	-.392	-.277	-.748	.767	1	
Si	.626	.527	.540	-.912	-.963	1

** correlation is significant at the 0.05 level (1-tailed)*
*** correlation is significant at the 0.01 level (1-tailed)*

Table 5. Correlation between the different water parameter using the Spearman R coefficient - 1 % of sodium alginate (Extract)

	DO	TAN	NO_3-N	NO_2-N	DIP	Si
DO	1					
TAN	-.988*	1				
NO_3-N	.563	-.683	1			
NO_2-N	.769	-.858	.961	1		
DIP	-.779	.866	-.957	-1.000**	1	
Si	.909	-.834	.166	.432	-.446	1

** correlation is significant at the 0.05 level (1-tailed)*
*** correlation is significant at the 0.01 level (1-tailed)*

The correlation between water quality variables in control tanks where shrimps were fed only with commercial feed showed no significant difference between the variables. But whereas in other tanks based on the bivariate correlation test, TAN had remarkable correlation with DO ($P < 0.05$), where shrimps were fed with Type 2, 3, 4 diet which indicates that the variation in TAN affects the dissolved oxygen.

Table 6. Correlation between the different water parameter using the Spearman R coefficient - 2 % of sodium alginate (Extract)

	DO	TAN	NO_3-N	NO_2-N	DIP	Si
DO	1					
TAN	.461	1				
NO_3-N	.970	.233	1			
NO_2-N	-.849	.077	-.952	1		
DIP	.957	.699	.858	-.659	1	
Si	-1.000**	-.480	-.965	.838	-.963	1

** correlation is significant at the 0.05 level (1-tailed)*
*** correlation is significant at the 0.01 level (1-tailed)*

Silicate was remarkably correlated with DO ($P < 0.05$) in tanks where shrimps were fed with Type 2 diet whereas remarkably correlated inversely with Type 5 diet ($P < 0.01$).Similarly, silicate was remarkably correlated with TAN ($P < 0.05$) in tanks where shrimps were fed with Type 2 diet whereas DIP was significantly correlated with NO_2-N ($P < 0.01$) in tank where shrimps was fed with Type 4 diet.

In the present study, the correlation test clearly shows the dependency between the various water monitoring variables. The result shows that NO_3-N was not significantly correlated with the other water variables. The role of NO_3-N was not significant. This might be attributed to the sodium alginate extract (natural source) used along with the feed.

In the recent years, the water quality has become one of the major factors that influenced the shrimp production process [30, 31]. The ability of farmers to endure the health of the stocked shrimp, and appropriately handling the culture environments to maintain the water quality variables within the specified limits is essential which leads to better shrimp development and growth. In the current study, the variables that most influenced the water quality were TAN, DO, DIP and silicate.

CONCLUSION

The present study documented that *Fenneropenaeus indicus* that was fed with diets containing sodium alginate showed beneficial effect on gut microflora and biochemical parameters. This study also provide essential information on the influence of supplemented diets on the water quality parameters.

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