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**EFFECTS OF REPLACING FISHMEAL WITH SEAGRASS *CYMODOCEA
SERRULATA* MEAL ON THE GROWTH AND BIOCHEMICAL COMPOSITION OF
WHITE LEGGED SHRIMP *PENAEUS VANNAMEI***

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ABSTRACT

Present attempt was made to assess the effects of replacing fish meal with seagrass *Cymodocea serrulata* meal on the growth and biochemical composition of White legged shrimp *Penaeus vannamei*. The investigations on the biochemical constituents and nutritive profile of the formulated feeds showed that seagrass could be the better replacement for fish meal in pelleted feeds. The seagrass used in our study did not seem to affect the palatability of the diet for a shrimp *P.vannamei*. The 21 days experiment result showed that the lipid and carbohydrate were found to be significantly higher in experimental feed. Whereas the insignificant difference was noted in protein content between control and experiment feeds. The survival, growth, content of protein and carbohydrate of shrimp were found to be significantly elevated in seagrass incorporated diet when compared to control. Hence, present study suggests that the seagrass *Cymodocea serrulata* can be used as an alternative source to replace high cost fishmeal in the diet of *P.vannamei*.

Key Words: Algae, Seagrass, Fish meal, *Cymodocea serrulata*, *Penaeus vannamei*

INTRODUCTION

Aquaculture remains one of the fastest food-producing sectors, accounting for almost half of total food fish and substitutes for wild fish and plants [1]. Feed is very large part of operating expenses in crustacean aquaculture [2] reaching a value as high as 50% [3]. An effective dietary protein source must satisfy an animal's requirement for both essential and non-essential amino acids [4]. Protein source is the largest cost and most important component in the aquaculture feeds. Generally, fish meal has been recognized as the major protein sources due to its abundance of essential nutrients. However, unstable production and higher price of fish meal have made it hard to meet the growing production demand of the aquafeed industry. Therefore, finding alternative protein sources to replace fishmeal in diets has become a global research priority. The aquaculture feed sector of India has made tremendous developments during the last two decades. At present about 20 million tones of manufactured aqua feed are being used in aquaculture sector, of which the majority is consumed in shrimp culture [5].

To sustain the aquafeed industry, a large part of the nutritional research has been focused on the search for alternative protein

source. Additionally, researchers have been focused on plant based diets provide essential fatty acids, phospholipids, sterols and carotenoids for growth, survival and normal metabolic function [3, 6]. The present study is aimed to analyse the effect of replacing fishmeal with seagrass *Cymodocea serrulata* meal on growth, survival and body composition of white legged shrimp *Penaeus vannamei*.

MATERIALS AND METHODS

Collection and identification of seagrass

The seagrass *Cymodocea serrulata* was collected from Muthukkuda, located along the Palk Bay in the Pudukkottai District of Tamil Nadu, Southeast coast of India (Lat. 9.8°N and Long. 79.1°E). The initial physico-chemical characteristics of water sample collected from seagrass sampling area were estimated according to Strickland and Parsons [7]. The values of physico-chemical parameters were given in **Table 3**.

Feed ingredients and preparation of feed

The feed ingredients such as soya bean, coconut oil cake, ground nut oil cake, tapioca flour, dry fish meal, green gram and egg were purchased from merchants; vitamin, mineral mix and cod liver oil were obtained from medical shops at Trichy. The

feed ingredients (**Table 1**) with dried seagrass *Cymodocea serrulata* powder was added in feed mix in known ratio of 10% and the composition of formulated diets were weighed out as per the standard formula and put into a mixer and homogenized the feed mix for 15 minutes, under room temperature. Feed ingredients were mixed well and brought into dough form. The feed mixer was loaded in the thermo stable trays and trays were kept in steaming cooker. The feed mixer was cooked at 95-100°C using steam cooker and kept for 15 minutes and then the feed were allowed to cool at room temperature. The vitamin mix and cod liver oil was added to the steam cooked feed mix and thoroughly homogenized in a dough mixer. In this feed mix, 10% of distilled water and egg albumin were added and homogenized for another 10 minutes to bring

the final mixture into a paste form. The feed mix paste was loaded in the manual hand pelletizer fixed with 3mm diameter disc and pellets were collected in an aluminium tray. Finally these pellets were dried in room temperature at 27°C for 48 hours.

Experimental conditions

A total of 200 shrimp *Penaeus vannamei* larvae were procured from Rank Marine Hatchery, Marakkanam, Villupuram district, Tamil Nadu, with proper aeration and then transferred to the laboratory. The larvae were stocked in sea water and the water sample was collected from culture tanks prior to the feeding experiment and analyzed for different parameters like pH, temperature, salinity, dissolved oxygen, ammonia, nitrate, nitrite, phosphate and silicate according to **Strickland and Parsons [7]** (**Table 2**).

Table 1: Composition of formulated diets

S. No	Composition	Control feed (g)	Experimental feed (g)
1	Dry fish meal	10	5
2	Seagrass powder	-	10
3	Ground nut oil cake	13	13
4	Coconut oil cake	15	10
5	Soya bean meal	15	10
6	Green gram	10	10
7	Wheat flour	10	10
8	Rice bran	12	12
9	Tapioca	5	5
10	Egg albumin	5	5
11	Cod liver oil	5	5
12	*Vitamin mix	3	3
	Total	100	100

*Becosules capsules composition

Folic acid, Vitamin B₁₂, Niacinamide, Calcium pantothenate, Pyridoxine, Ascorbic acid, Riboflavin, Thiamine, Biotin

Table 2: Physico-chemical parameter in shrimp culture water

S. No	Parameters	Values
1	Water temperature (°C)	26±0.58
2	pH	8.2
3	Salinity (ppt)	15
3	Dissolved oxygen (mg/l)	3.5±0.33
4	Nitrate (µM/l)	0.09±0.01
5	Nitrite (µM/l)	0.063±0.024
6	Phosphate (µM/l)	0.39±0.02
7	Ammonia (µM/l)	0.31±0.004
8	Silicate (µM/l)	1.42±0.002

The shrimp larvae were acclimatized for 3 days period to feeding experiment. During acclimatization, shrimp were fed with boiled egg albumin and control feed without seagrass. Water was routinely changed every-day in order to maintain a healthy environment for the shrimp apart from providing artificial aeration. This ensures sufficient oxygen supply for the shrimp and an environment devoid of accumulated metabolic wastes. Initially 100 numbers of shrimp larvae was stocked and fed twice a

day with control and experimental diet for about 21 days. The feed was given at 10% of shrimp body weight. The unconsumed feed, if any, was siphoned out during renewing the culture water. Likewise, faecal matter was also siphoned out prior to the next feeding.

The growth parameters of the experimental shrimp larvae were assessed by measuring their body weight at 10 days interval and survival was calculated according to the below formulae.

$$\text{Survival (\%)} = \frac{\text{Number of lives shrimp}}{\text{Number of stocking shrimp}} \times 100$$

Biochemical Analyses

The biochemical composition such as total protein [8], total carbohydrate [9], total lipid [10], moisture [11] and ash [12] were estimated in both formulated feeds and the shrimps according to the standard methods.

Statistical Analyses

The given data were expressed in terms of the mean of three replicates ± SD (standard deviation). Length and weight parameters in *P.vannamei* were analyzed by

paired sample t-test at $p < 0.05$ by using SPSS Inc. program version 14.

RESULTS AND DISCUSSION

Seagrasses are the only flowering plants living under water in the photic zone of coastal areas. They form beds of mono specific or mixed communities and can support very high biodiversity [13, 14]. They provide food for invertebrates (urchins) and vertebrates (as fishes, turtles and Dugong), and constitute habitat, shelter and nursery for

fishes and crustaceans [15, 16] Seagrasses are important source of primary production [17, 18]. Seagrasses take up nutrients from the sediments, transporting them through the plant and releasing them into the water column through the leaves.

Low nutrient values were observed in study area due to the absorption of nutrients by the seagrass. Nutrients essential to fish are the same as those required by other animals. In the present investigation, water quality parameters (Table 1) such as water temperature ($30 \pm 0.58^\circ\text{C}$), pH (8.3 ± 0.09), salinity (32 ± 1 ppt), dissolved oxygen (2.77 ± 0.33 mg/l), nitrate (0.811 ± 0.01 $\mu\text{M/l}$), nitrite (0.081 ± 0.024 $\mu\text{M/l}$), phosphate (0.25 ± 0.02 $\mu\text{M/l}$), ammonia (0.31 ± 0.004 $\mu\text{M/l}$), silicate (2.4 ± 0.06 $\mu\text{M/l}$), chlorophyll 'a' (1.99 ± 0.011 mg/ml) and chlorophyll 'b' (2.24 ± 0.073 mg/ml) were estimated and results were given in Table 3.

Fish meal is one of the most expensive ingredients in prepared fish diets. Fish nutritionists have tried to use less expensive plant protein sources to partially or totally replace fish meal. Finding alternative protein sources to replace fishmeal in fish feed is important if the growth of the aquaculture industry is to be sustained. While formulating the feed vitamin mixture is added as it was clearly demonstrated that

there are some interactions between vitamins. This is an important factor to be considered while formulating the feed with vitamin mixture. In the present study, proximate composition of control and experiment diets showed (Table 4) insignificant variations in protein 24.39 ± 3.58 % in control while in experiment group it was reported as 23.08 ± 0.57 % levels. However the lipid was 11 ± 1 % in control group and 16 ± 0.2 % in experimental group and carbohydrate was 48.44 ± 2.42 % in control and 73.35 ± 3.57 % in experimental group. The amount of ash (25 %) and moisture content (28 %) of the experimental feed was found to be higher than the control feed (ash: 24 % and moisture: 24 %) contents showed significant elevations in experimental diets. It indicates that, the selected experimental seagrass has considerable nutrients for shrimp feed formulation.

The biochemical composition of control and seagrass incorporated feed fed experimental shrimps showed significant variation. The protein in control group was 11.11 ± 0.69 , 13.91 ± 0.01 , 13.99 ± 1.39 while in experimental group it was 11.11 ± 0.69 , 20.36 ± 0.56 , 21.67 ± 2.05 on initial, 10th day and 20th day of experiment respectively. The amount of lipid in shrimp *P. vannamei* showed sudden increase in

control group when compared to the experimental group. Initial lipid was 13.5 %. The recorded larval lipid content was 15 % and 24 % in control group whereas in experimental group it was 17 % and 20 % on 10th and 20th day of experiment respectively. Seagrass feed has been found to contain high amount of carbohydrates in shrimp *P. vannamei* control group (26.27 ± 0.72 , 29.43 ± 8.75 , 76.46 ± 7.54 %) while in experimental group it was 26.27 ± 0.72 , 29.27 ± 7.31 , 75.42 ± 7.38 % for initial, 10th and 20th day respectively. Hence use of carbohydrate rich plant source would be a viable option to replace protein sparing effect in the diets. The addition of small amount of binder to the diet is another approach that has been used to enhance feces stability [19-21].

Using balanced formulations based on alternative protein sources, primarily of plant origin, has resulted in an improvement in the overall nutritional quality of practical diet formulations [22, 23] as well as considerable reduction in formulation costs. In this experiment, seagrass has been used as a partial replacement of fish meal in the feed for shrimp PL. The biochemical composition of the feeds showed higher protein and carbohydrate content in the seagrass formulated feeds as they are rich in polysaccharides. Carbohydrates are

considered to be the first among the organic nutrients can be utilized to generate required energy. Lipids are primarily included in formulated diets to maximize their protein sparing effect [24] by being a source of energy. In this study the experimental shrimps showed drastic increase in lipid content after they got acclimatized to the feeding experiment. Dietary lipids are known to play an important role in animal nutrition as they provide energy, maintain the structural integrity of biological membranes and function as precursors of important steroids [25].

The survival rates obtained in this study with the formulated diets seem to indicate that there were no overwhelming negative effects in utilization of nutrients by the shrimp *P. vannamei*. Higher survival rate was reported in experimental shrimps which could be due to the growth promoting substances present in the seagrass. The growth parameters such as length of shrimp *P. vannamei* in control group was 1.66 ± 0.06 cm, 1.87 ± 0.06 cm and 3.17 ± 0.07 cm and in experimental group it was 1.66 ± 0.06 cm, 2.03 ± 0.15 cm and 3.6 ± 0.08 cm for initial, 10th and 20th day respectively. Overall length was found to be increased gradually from 10th day to 20th day in both the groups whereas in experimental groups higher

growth was achieved. The measurement of weight in shrimp *P. vannamei* in control group was 0.02 ± 0.002 gm, 0.04 ± 0.002 gm, 0.20 ± 0.04 gm while in experimental group it was 0.02 ± 0.002 gm, 0.05 ± 0.003 gm, 0.29 ± 0.02 gm on initial, 10th and 20th day respectively. Overall weight was found to be increased drastically from 10th day onwards in both the groups (Table 6). However, the weight was comparatively higher in experimental group.

Growth is function of both nutritional quality and the rate of consumption, among

other things [26]. The effects of the nutritional diets on the growth and survival of shrimp *P. vannamei* showed better survival rates and high protein profile of the experimental groups. In this experiment, it is very clear that the seagrass meal was most effective for fishmeal replacement and the feeds were found to possess high nutritional value due to its high protein content and carbohydrate in low cost, availability and steady supply as compared to other plant protein sources.

Table 3: Physico-chemical parameters in Muthukkuda water samples

S. No	Parameters	Values
1	Water temperature (°C)	30±0.58
2	pH	8.3±0.09
3	Salinity (ppt)	32±1
3	Dissolved oxygen (mg/l)	2.77±0.33
4	Nitrate (µM/l)	0.811±0.01
5	Nitrite (µM/l)	0.081±0.024
6	Phosphate (µM/l)	0.25±0.02
7	Ammonia (µM/l)	0.31±0.004
8	Silicate (µM/l)	2.4±0.06
9	Chlorophyll 'a' (mg/ml)	1.99±0.011
10	Chlorophyll 'b' (mg/ml)	2.24±0.073

Table 4: Proximate composition of control and experiment diets

S. No	Biochemical in formulated feed	Control feed %	Experiment feed %
1	Protein	24.39±3.58	23.08±0.57
2	Lipid	11±1	16±0.2
3	Carbohydrate	48.44±2.42	73.35±3.57
4	Moisture	24	28
5	Ash	24	25

Table 5: Analysis of biochemical composition of shrimps

S. No	Biochemical Constituents	Initial Shrimp	Control Shrimp		Experiment Shrimp	
			10 th day	20 th day	10 th day	20 th day
1	Protein	11.11±0.69	13.91±0.01	13.99±1.39	20.36±0.56	21.67±2.05
2	Lipid	13.1± 0.4	15.9± 0.9	24.15±0.15	16.9± 0.1	20.6±0.6
3	Carbohydrate	26.27±0.72	29.43±8.75	76.46±7.54	29.27±7.31	75.42±7.38
4	Moisture	25±0.7	28.2±0.3	34.8±0.4	28.5±0.15	36±0.65
5	Ash	22±0.2	22.8±0.75	24.8±0.2	22.95±0.75	23.8±0.85

Table 6: Measurement of length and weight in white leg shrimp *P. vannamei*

Morphometric measurement	Initial day		10 th day		20 th day	
	control	experiment	control	experiment	control	experiment
Survival %	100	100	89.2±2	98.3±1.5	87.5±1.5	96±2
Length (cm)	1.66 ±0.06	1.66 ±0.06	1.87 ± 0.06	2.03 ± 0.15	3.17±0.07	3.6 ± 0.08
Weight (gm)	0.02 ± 0.002	0.02 ± 0.002	0.04 ± 0.002	0.05 ± 0.003	0.20±0.04	0.29±0.02
Length gain (cm)	0.0	0.0	0.27±0.055	0.042±0.057	1.57±0.067	1.78±0.084
Weight gain (gm)	0.0	0.0	0.016±0.002	0.02±0.003	0.25±0.13	0.26±0.19
Length comparison between the groups (Con and Exp)				t value - 2.278 P value - 0.826	t value - 4.676 P value - 0.44	
Weight comparison between the groups (Con and Exp)				t value - 3.003 P value - 0.035	t value - 5.297 P value - 1.55	

* denotes significant at 5% level.

CONCLUSION

Seagrass have the tendency to absorb nutrients from the environment and hence serve to be a natural nutrient for fish and shrimp, providing habitat for various marine organisms. Although ecological work related with seagrass is numerous there is few previous works available on the use of seagrass as dietary protein source for shrimps. So, in the present study, the replacement of fish meal diet up to 10% with *Cymodocea serrulata* was carried out. In this research trial, an incorporated diet containing seagrass was found to be the optimal dietary requirement for shrimp *Penaeus vannamei* which showed better growth and survival. It was found that the ingredients of these treatments have excellent nutritional value with high palatability.

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