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Partial replacement of fishmeal with seagrass *Syringodium isoetifolium* in formulated diets to evaluate the growth performance of freshwater ornamental fish *Poicilia reticulate*

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ABSTRACT

The present study deals with the effect of different concentration of *Syringodium isoetifolium* in the experimental diets were formulated by replaced fishmeal at 10%, 20%, 40%, 60%, 80% were prepared in pellet form and fed to the fish, *Poicilia reticulate* to evaluate changes in the growth and food conversion ratio. There is no previous works available on the use of Sea grass as dietary protein source for fishes. The tolerance towards this formulated diet was determined by low mortality rate (12%) and specific growth rate (1.16%) as well as better feed conversion ratio (1.26) was observed in incorporated diet containing 80% *Syringodium isoetifolium*. The growth performance of fish fed with varying concentration of sea grass, *S. isoetifolium* was statistically analysed and there was no significant difference ($P > 0.05$) between control, 10%, 20%, 40%, 60%, 80%. As the concentration of sea grass increases in formulated diet, the better growth performance was observed in fishes. The study proves that 80% of sea grass, *Syringodium isoetifolium* found to be good replacer for dietary fishmeal.

Keywords: *Syringodium isoetifolium*, fish, formulated diet, *Poicilia reticulate*, fishmeal, growth performance

INTRODUCTION

Aquaculture feed form the most expensive input and 57-87% of the total recurring expenditure (Nandeesha, 1993). The cost of fish feed through use of these alternative and less conventional raw materials would be ideal for protection farm based aqua feeds (Jana *et al* 1998). The past several years, one of the main directions in improving fish feeds has been the search for protein source alternatives to fish meal and determining their nutritional suitability in diets. The production and trade ornamental fish is a profitable alternative in the aquaculture sector. Freshwater and marine species have been used successfully in the aquarium fish trade such as discus (*Symphysodona equifaciatus*), guppy (*Poecilia reticulata*), swordtail (*Xiphophorus helleri*), molly (*Poecilia sphenops, Poecilia latipinna*) and goldfish (*Carassius auratus*) (Chong *et al* 2003 and Blom *et al* 2000). Despite the economic importance of this sector, the nutritional information for ornamental fish is scarce and often few or even no data of the nutritional requirements is available (Sales *et al* 2003). Many authors have studied the impact of replacement of fish meal on growth of fishes mostly in edible, but studies related to dietary formulation of fish performance and reproduction in ornamental fishes are scanty. So, this present study deals with the effect of the partial substitution of fishmeal with seagrass

Syringodium isoetifolium to evaluate the growth performance of the freshwater ornamental fish, *Poicilia reticulate.*

MATERIALS AND METHODS

Study area & collection of seagrass

Fresh *Syringodium isoetifolium* was collected from Thondi (Lat. 9°45'N and Lang. Most 79°3'E) is situated 40 km south of Manelmalkudi and 45 km north of Devipattinam, Tamilnadu, South-East coast of India. The collected seagrass was washed with seawater twice. The samples were brought to the laboratory in plastic bags and then again washed with distilled water to remove potential contaminants and epiphytes. The leaves were separated and dried in the shade for 10-14days. The dried leaves were sufficiently powdered using an electric blender and were stored in the refrigerator for further use (Sundaravadivel *et al* 2012).

Collection of *Poicilia reticulate*

Juvenile Guppies, *Poicilia reticulate* weighing around 230 to 250 mg at the beginning of the feeding trials were used in this study. These fish were obtained from a local fish dealer at the Kolathur, Chennai, India. They were transported in plastic oxygenated cover to Laboratory. Fish were acclimated under the experimental conditions for a period of 10 days. Individual size of fishes range from 228.67±67 to 267.33±18.04 mg was recruited from the stock and five fishes were placed in each of 18 round plastic troughs.

The fishes were acclimatized for a period of ten days under laboratory condition. During the acclimatization period as well as experimental period, water temperature ($29^oC \pm 1^oC$), chlorinity (1.38ppt), salinity (2.53ppt), pH (7.5), dissolved oxygen (3.67mg/l), CO_2 (20mg/l) were maintained constantly (AOAC,1980).

Six formulated diets were prepared as pelleted form by using the following ingredients mentioned below in the table-1. Each group of test animals were fed with 7% to 10% of body weight of fish twice a day in the morning (9.00am) and evening (4.00pm). The unfed and

Table-1: Diet Formulation

INGREDIENTS	Control	SI1-10%	SI2-20%	SI3-40%	SI4-60%	SI5-80%
Fish meal (mg)	40	36	32	24	16	8
Groundnut Oilcake (mg)	22	22	22	22	22	22
Soyabean meal (mg)	10	10	10	10	10	10
Ricebran (mg)	18	18	18	18	18	18
Tapioca flour (mg)	06	06	06	06	06	06
Vitamin and Mineral *(Supradyn tablet) (mg)	02	02	02	02	02	02
Cod Lliver Oil (mg)	02	02	02	02	02	02
Syringodium isoetifolium (mg)	0	04	08	16	24	32

SI - *Syringodium isoetifolium;* *Supradyn- 02 ⟶2 tablets

Table-2. Proximate Composition of Ingredients Used in Formulated Diets

Ingredients	Protein (%)	Carbohydrates (%)	Lipid (%)	Ash (%)	Energy (J/mg)
Fish meal	19.17	4.56	29.62	19.39	361.52
Groundnut oil cake	48.9	6.70	10.7	11.5	318.7
Rice bran	15.7	20.40	13.4	12.5	265
Soya bean Meal	49.56	8.85	1.37	18.64	245.97
Tapioca Flour	14.9	43.7	0.1	8.9	235.3
Test Ingredient (Seagrass)					
Syringodium isoetifolium	42.321	39.297	19.21	31.112	499.3

Table-3: Proximate Composition of Formulated Diets.

Conc	Feed Types	Protein (%)	Carbohydrates (%)	Lipid (%)	Ash (%)	Energy J/mg
Control	C	17.04	12.51	27.16	13.28	362.64
10%	SI1	16.54	28.26	19.45	12.99	291.34
20%	SI2	19.89	43.66	20.34	15.7	391.09
40%	SI3	15.83	49.54	20.92	8.2	449.71
60%	SI4	16.02	39.90	20.45	20.3	390.6
80%	SI5	0.47	49.86	20.32	10.2	464

Table-4. Growth performance of Poicilia reticulate fed on formulated diets containing *Syringodium isoetifolium* at different concentration.

DIETS	INITIAL WEIGHT (mg)	FINAL WEIGHT (mg)	*GROWTH (mg)	*GROWTH RATE (mg)	*SPECIFIC GROWTH RATE (SGR)%	*FOOD CONVERSION RATIO (FCR)	*MORTALITY RATE (MR)%
CONTROL	264.66± 13.61	379.33± 12.86	114.67± 1.15	43.41± 2.51	0.9±0.05	2.32± 0.09	21
SI1	267.33± 18.04	389.33± 19.01	122±2	45.76± 2.78	0.94± 0.05	2.15± 0.04	18
SI2	228.67± 13.61	365.33± 13.32	136.67± 1.15	49.87± 0.56	1.17± 0.06	1.51± 0.02	19
SI3	274±2	414.67± 4.16	140.67± 2.31	50.08± 2.79	1.01± 0.01	1.35± 0.05	16
SI4	257.33± 11.72	386± 10.58	128.67± 1.15	58.99± 1.66	1.01± 0.04	1.33± 0.04	14
SI5	245.33± 5.03	390±4	144.67± 1.15	59.91± 3.64	1.16± 0.03	1.26± 0.03	12

Values are expressed as Mean ± SD of triplicate groups of five fishes

*Growth (mg) = Final weight of fish (F) – Initial weight of fish (I)
*Growth rate (GR) % = Growth (mg)*100/Initial weight of fish
*Specific growth rate (SGR)% = (Ln F – Ln I)*100/Experimental duration (days)
Where, Ln = natural logarithm.
*Food conversion ratio (FCR) = Dry food consumed (mg)/Wet weight gain (mg)
*Motality rate (MR)% = No.of dead fishes in each sample *100/No.of total dead fishes in all samples

faeces were collected daily by manual siphoning method. The experimental duration lasted for 41days and the water was changed in each experimental setup in the morning for every two days, before first feeding of the fishes. Care was taken to observe keenly the growth of fishes and the final weight of the each fishes were weighed at 41st day to determine the growth. During the course of experiment, the number of fishes died in each replicates of groups was also noted. At the end of the feeding trial, the fishes were sacrificed and the live and dry weight were recorded (Sundaravadivel *et al*, 2012).

Quantitative Analysis

Six diets with formulated pelleted diets of six different concentration of *Syringodium isoetifolium* (Control, 10%, 20%, 40%, 60%, 80%) were formulated using low cost ingredients. The dietary ingredients of each diet were mixed for about 10 min. Cod liver oil was gradually added to the mixture and the ingredients were mixed for another 5 min. Subsequently, a sufficient quantity of water was added to the mixture, which was then blended for another 5 min and extruded through a pelletizer having 0.1 mm diameter. The freshly prepared moist pellets were shade dried for few

hours and fed to the fish at (7-10) % of their total body weight. Carbohydrates (Hodge and Hofriter, 1962), Protein (Lowery *et al*, 1951), Lipid (Folch *et al*, 1957) were estimated for ingredients and formulated feeds using HPLC. The ash content was determined by incinerating a known dry sample in a muffle furnace at 560^{o}C for 8hours using Muffle furnace (Paine, 1964).

RESULT

The proximate composition of ingredients and percentage composition of formulated diets are given in table 1 & 2 respectively. The proximate composition of formulated diet is given in the table-3. The *Syringodium isoetifolium* 80% (SI5) incorporated feed consists of higher protein (20.47%) and low dietary protein (15.83%) was observed in 40% (SI3) incorporated diet. The high carbohydrate content (49.86%) was observed in the feed 80% (SI5) and the low dietary carbohydrate (12.51%) was observed in the Control (C) diet. The lipid content was more (27.16%) in Control(C) and low lipid content (19.45%) was observed in 10 % (SI1) incorporated feed (Figure-1).

Figure-1. Graphical representation for Proximate composition of formulated diet containing *Syringodium isoetifolium.*

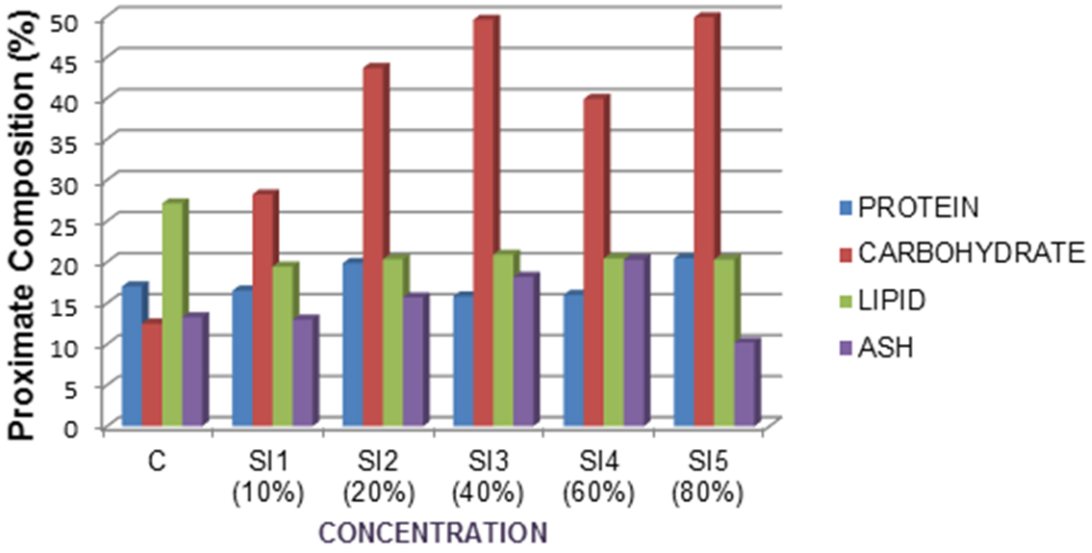
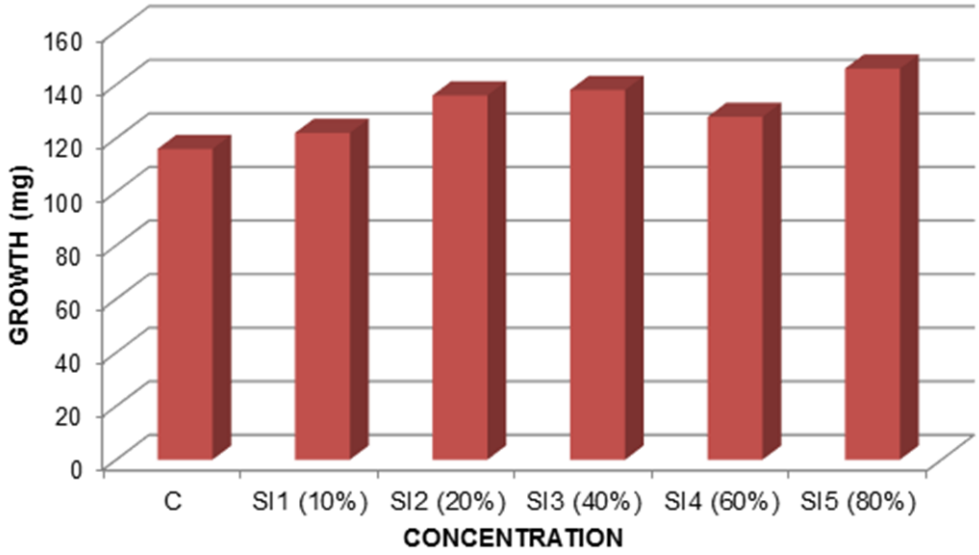


Figure-2. Graphical representation of effect of formulated diet *S. isoetifolium* in varying concentration in the growth performance of *P. reticulate*



The growth performance of *Poicilia reticulate* fed with formulated diets containing *Syringodium isoetifolium* is given in the table-4 and in Figure-2. Among the six varying concentration, the highest growth rate 59.91±3.64 (%) was observed in *Poicilia reticulate* fed with 80% (SI5) incorporated diet. The lowest growth rate 43.41±2.51 (%) was noticed in Control (C) diet (Figure-3).

Among the varying concentration of *Syringodium isoetifolium*, good growth was noticed with low FCR when 80% of fish meal was replaced by 80% *Syringodium* meal (feed type SI5) in the Figure-4. The good growth of fish fed on SI5 incorporated diet may be due to the presence of seagrass in the diet containing all the dietary essential nutrients like proteins, carbohydrates, vitamins and minerals, etc. The growth rate of *Poicilia reticulate* in the control diet was significantly less when compared with diets containing varying concentrations of *Syringodium isoetifolium.*

Figure-3. Graphical representation of effect of formulated diet *S. isoetifolium* in varying concentration in the growth rate of *P. reticulate.*

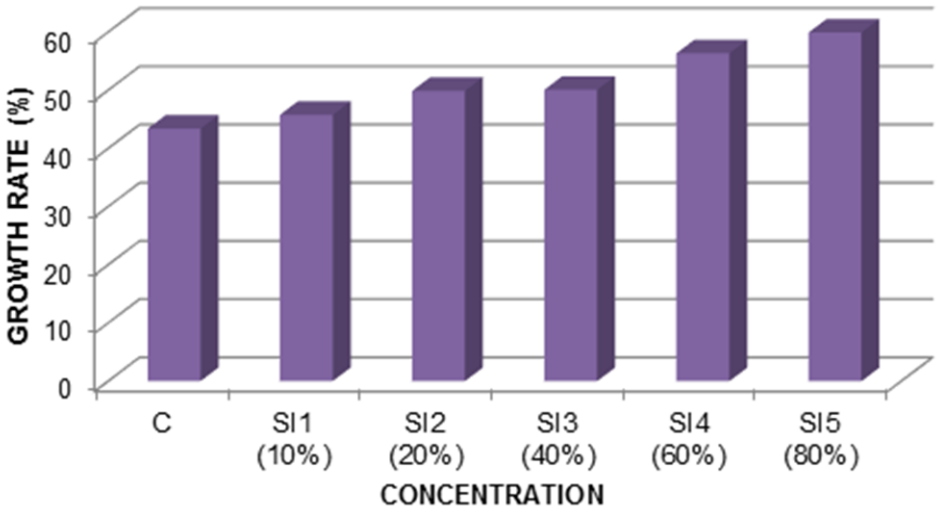
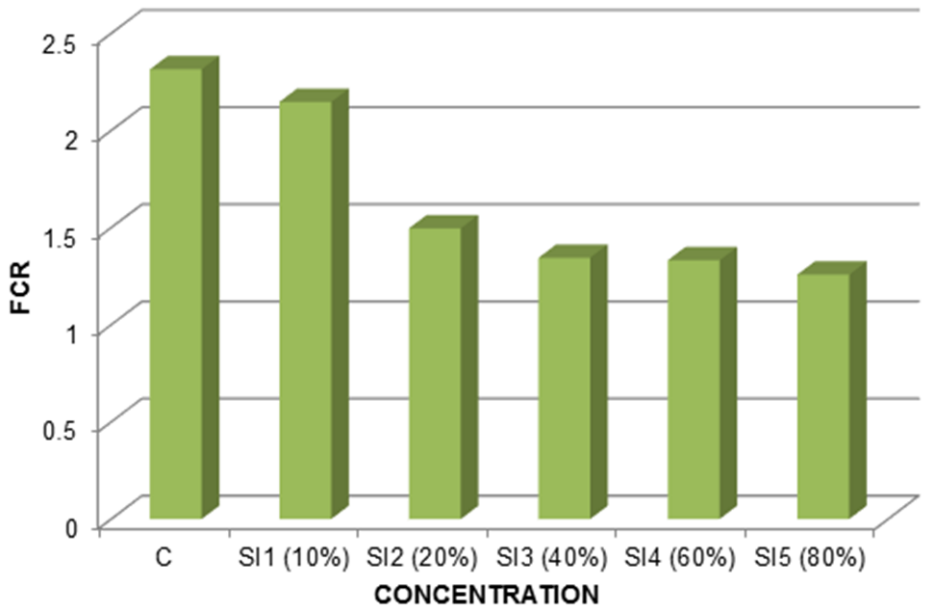


Figure-4. Graphical representation of effect of formulated diet *S. isoetifolium* in varying concentration in the FCR of *P. reticulate*



During the feeding trial, the fish readily accepted all the diets and mortality rate ranged from (12-21)%. The highest specific growth rate (1.16±0.03%) was observed in fish fed with diet SI5 (49.86% carbohydrate level). Values of SGR ranged from 0.90–1.16% in all experimental diets. Weight gain (%) increased as carbohydrate levels rose from 12.51–49.86% in the formulated diets (Figure-1).

Feed conversion ratio (FCR) was better (1.26-1.35) among the groups (diets SI3, SI4, and SI5) where SGR (1.01–1.16 %) and weight gain were also higher. Better SGR (1.16±0.03%) and FCR (1.26±0.03) values were found in diet SI5 (Figure-4). There were significant differences in the proximate whole body composition after the feeding experiment. Fish fed Control diet had higher crude protein (CP) content (31.39 %) in their carcass. Then fish fed protein levels at 10 % (30.57% CP), 20 % (27.61 % CP), and at 40 % (28.87 % CP) respectively, however there is significant differences in the Crude protein body samples were also observed.

The graphical representation shows the proximate composition of formulated diet, growth, growth rate, FCR in the figure- 1,2,3&4.

DISCUSSION

Growth is a function of both nutritional quality and the rate of consumption, among other things (Stickney 2000). Several authors have studied the effects of different nutritional diets on the growth and survival of different fish species. But there is no previous works available on the use of Seagrass as dietary protein source for fishes. So, in the present study, the replacement of fishmeal diet upto 10% to 80% with *S.isoetifolium* was carried out. In this research trial, an incorporated diet containing 80% Seagrass, *Syringodium isoetifolium* was found to be the optimal dietary requirement for guppies.

Many fish farmers and ornamental hobbyists buy the bulk of their feed made commercially. However, small quantities of specialized feeds are often needed for experimental purposes, feeding difficult-to maintain aquarium fishes, larval or small juvenile fishes, and brood fish conditioning, or administering medication to sick fish. Small-ornamental fish farmers with an assortment of fish require small amounts of various diets with particular ingredient. It is not cost effective for commercial manufacturers to produce very small quantities of specialized feeds. Most feed mills will produce custom formulation of more than one ton, and medicated feeds are usually sold in 50 pound bags. Small fish farmers, hobbyists and laboratory technicians are therefore, left with the option of buying large quantities of expensive feed, which often goes to waste. Small quantities of fish feeds can be made-quite easily in the laboratory, class room or at home with common ingredients and simple kitchen or laboratory experiment. Nutrients essential to fish are the same as those required by other animals. These include water, protein, lipids, carbohydrates, vitamins and minerals (Abovei and Ekubo, 2011; Royes and Chapman, 2009).

In natural environment, fish have developed a wide variety of feeding specialization (behavioural, morphological and physiological) to acquire essential nutrients and utility varied food sources.

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. Of all the plant protein feedstuff, soybean meal is considered to be the most nutritious and is used as the major protein source in many fish diets (Lovell 1988). Determination of palatability of a feed ingredient is an important criterion in the evaluation of that ingredient for fish. The digestibility of a particular feed ingredient reflects in growth of fish. Digestibility depends upon various factors like nature, dietary component, and type of nutrient and level of inclusion. (De Silva *et al*, 1990).

The details of formulation and processing of fish diets have been studied by Cho (1985), Robinson (1991), Halver (1980) and Lovell (1989). Bulletin of Food and Agriculture Organization

(FAO) and United Nation of Development Programmed (UNDP) had also mentioned about feed formulation. Finding alternative protein sources to replace fishmeal in fish feed is important if the growth of the aquaculture industry is to be sustained. Soybean meal is one such potential alternative. But in this experiment, it is very clear the seagrass meal are most effective alternative for fish meal in feeds with high nutritional value due to its high protein content and excellent carbohydrate content in low cost, availability and steady supply as compared to the other plant protein sources. Essential or indispensable amino acids (EAAs) cannot be synthesized by fish and often remain inadequate but are needed for growth and tissue development. 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.

The effectiveness of plant origin material in replacing fishmeal may also depend on the technology used for pellet preparation. Extrusion is an effective process to increase the nutrient availability of plant meals (Pongmaneerat, 1993; Somaiah *et al*, 2014). Thus, the nutritional value of seagrass concentrate may increase after extrusion. Seagrass, *Syringodium isoetifolium* was successfully replaced by fish meal at the level of 80% with effective growth performance in *Poicilia reticulate.*

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