Effect of diets containing medicinal plant ingredients on growth performance of red tilapia

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Abstract

Present study was carried out to ascertain the effect of diets supplemented by three medicinal plant ingredients (Kathurumurunga: *Sesbania grandiflora* (T_1), Beli: *Aegle marmelos* (T_2) and Garlic: *Allium sativum* (T_3) on growth performance of *Oreochromis* sp. (red tilapia) and water quality parameters. Red tilapia fry (*Oreochromis* sp.) were used for the experiment (n=360). Fish were stocked in three circular fiber glass tanks to acclimatize for one week. Circular fiberglass tanks were treated with ingredients of *Sesbania grandiflora*, *Aegle marmelos*, *Allium sativum* containing diets and control diet. Experimental design was Complete Randomized block design with triplicate; hence twelve experimental indoor fiberglass tanks were utilized. Thirty red tilapia fries weighing with 0.3052(±0.0086) g were stocked in each tank and were fed with 5% of their body mass twice per day for three-month period. Physicochemical parameters such as pH, conductivity, temperature, Dissolved Oxygen (DO), Biological Oxygen Demand (BOD_5), nitrate and ortho-phosphate concentrations were recorded every second week. Fish in garlic treated (T_3) and control (C) tanks were shown higher growth rates. Condition factor of fish in T_1, T_3 and control tanks was significantly higher ($p<0.05$) than T_2 tanks. Water quality parameters such as BOD_5, conductivity, nitrate and ortho-phosphate concentrations in control tanks were higher than treated tanks. All physicochemical parameters but pH were not significantly different ($p<0.05$) in experimental groups. Diets with medicinal plant ingredients have the ability to maintain good water

quality in the fish culture system. *Sesbania grandiflora, Allium sativum* treated tanks and control tanks showed significantly higher growth performances than *Aegle marmelos* (T_2) treated tanks. The present study concludes that fish in control and garlic-treated tanks shows higher growth rates than other fish in the experiment and, further investigations with a large fish are recommened to validate the present results.

Keywords: Condition Factor, Feed Ingredients, Garlic, Medicinal Plant,

Introduction

Feeding and disease control which are the major constraints in aquaculture sector involve comparatively high cost, affecting profitability and sustainability. Improving fish growth performance and disease control & resistance in cultured organisms are the major challenges faced by fish culturists. Fish meal that is compatible with the protein requirement of fish is considered the best ingredient among commonly used feed ingredients (Alam et al., 1996).

In order to sustain the aquaculture industry, alternative protein sources should be found to replace fish meal in fish feed because of its high cost (Francis et al., 2001; Tacon et al., 2003). Higgs et al. (1995) have also shown that replacement of fish meal with cheaper ingredients of plant origin is necessary, because of rising cost and uncertain availability of fish meal. Fish nutritionists have tried to use cheap plant protein sources partially or totally to replace the fish meal (Lovell, 1988).

World Health Organization encourages use of medicinal herbs and plants to substitute or minimize the use of chemicals. Medicinal plants consist of different active ingredients such as alkaloids, flavonoid pigments, phenolics, steroids and essential oils that lead to change performances in organisms such as antistress, growth promotion, appetite stimulation, immunostimulation, etc (Levic et al., 2008).

Sesbania grandiflora commonly known as vegetable hummingbird is a small tree in the genus *Sesbania* of the legume family. Edible flowers and leaves of *S.*

grandiflora are common food item of the people in Southeast and South Asia. Also, *S. grandiflora* have ahown antioxidant, anti-inflammatory, antibacterial, and antiuroithialic activity (Gomase et al., 2012). Scientific studies have confirmed many ethnomedicinal uses of *Aegle marmelos* (bael) such as free radical scavenging, antioxidant, inhibition of lipid peroxidation, antibacterial etc. (Manjeshwar et al., 2011). Studies have revealed that *Aegle marmelos* acquires a variety of nutritional elements like large group of phytochemicals which include phenolic acids, flavonoids, alkaloids, tannins, and coumarins. Other than these, amino acids, fatty acids, wide range of organic acids, minerals, carbohydrates, vitamins, and fibers make *Aegle marmelos* a highly nutritious fruit with immense health benefits (Tanmay et al., 2020). *Allium sativum* (Garlic) is an important vegetable extensively cultivated in many countries. It is used as food for humans as well as some animals and as a remedy for several diseases. *A. sativum* has been used to enhance the growth and resistance for disease in many varieties of livestock and fish. It has been reported significant weight gain, increase in feed efficiency, protein efficiency ratio and specific growth rate in Nile tilapia when fed with a diet containing 30 g/kg garlic powder (Shalaby et al., 2006). Research that has been carried out to investigate the effect of aqueous garlic extract on the macromolecular synthesis of *Candida albicans* found that protein and nucleic acid synthesis were inhibited to the same extent as growth, but lipid synthesis was completely prevented. Some people use garlic as an immune system booster compared to Vitamin C (Adetumbi et al., 1986).

Objectives of the present study were to investigate the effect of diets containing medicinal plant ingredients on the growth performance of red tilapia (*Oreochromis* sp.) and asses the water quality in the experimental tanks.

Materials and Methods

Experimental Design

Experiment was carried out in the Animal house of the Department of Zoology, Faculty of Science, University of Ruhuna, Matara, Sri Lanka. Fries of red tilapia

(*Oreochromis* sp.) were obtained from breeding ponds at Tilapia Breeding Centre, Udawalawa, Sri Lanka. The fish fries were stoked in re-circulating water tanks and allowed to acclimatize for one week. The fish were fed with control diet for 07 days, prior to start the experiment. Randomly selected fish fries from the rearing tank were weighed and transferred to the experimental tanks one week before starting the experiment to acclimatize them to experimental conditions.

Four different diets were tested in triplicate, and the experiment set-up was randomly assigned. Thirty red tilapia fries with an average initial Body Weight (BW) 0.3052 ± 0.0086 g were introduced into the each treatment group. At the beginning and end of the experiment, each acclimatized fish was individually weighed using a digital scale and total length was measured using a measuring ruler.

Preparation of Experimental Diets.

The experimental diets were consisted of one control diet (C) and three test diets (T_1, T_2, and T_3). The control diet contained fish meal as the main crude protein (CP) source. In the three test diets tested, 35% of the weight in control diet was replaced by alternative medicinal plant ingredients.

The medicinal plants, *Sesbania grandiflora* (Kathurumurunga), *Aegle marmelos* (Beli) and *Allium sativum* (Sudu loonu/ Garlic) were collected from Ambalangoda in Galle district. The collected plant parts were washed with distilled water and air dried under sun light. The dried medicinal plant parts were finely ground by using a grinder. Grinded materials were sieved through a nylon sieve (100 µm) in order to remove plant fibers. Soya bean, coconut meal and wheat flour were used as other plant protein sources.

Selected medicinal plant ingredients and other protein-containing ingredients were subjected to proximate analysis prior to feed formulation. Proximate protein composition of medicinal plant ingredients and other ingredients. i.e., fish meal,

soya bean, coconut meal and wheat flour were measured using a Kjeldahl Analyzer; Model VELP Scientific UDK 139.

Protein level of 40% was selected to formulate test diets and the control diet. Control diet with 40% protein was prepared using fish meal, soya bean, coconut meal, wheat flour and other diet formulating ingredients. From the weight, 35% of control diet was substituted with medicinal plant ingredient to formulate the test diets. The diets were made into a form of strings using a string hoppers making machine having a perforated diameter of nearly 2.5 mm. Dried pellets were broken manually into very small pieces depending on the size of the experimental fish. All the dried pellets were packed in polyethylene bags and stored in a refrigerator at $4^{o}C$ until they were used in the experiment. Fish were fed approximately 5% of their body weight twice a day for 3 months period.

Weight and length measurements were taken every fourteen days. After first two weeks, length measurements were taken for randomly selected 15 fish. Condition factor for each fish was calculated. Tanks were cleaned by siphoning out faecal matter and uneaten feeds once in two weeks.

The following indices were used to determine growth performance of fish.

Relative Growth Rate = Weight (g) or Length Increment during the time interval (cm) / Initial Weight (g) or Length (cm)

Condition factor = $[W/ L^3] \times 100$

[Where: W = Weight increment of fish (g); L = Length increment of fish (cm)]

Water Quality Measurements

Physicochemical parameters pH, conductivity, temperature, Dissolved Oxygen (DO), Biological Oxygen Demand (BOD_5), nitrate and ortho-phosphate concentrations were measured once in two weeks.

Statistical Analysis

All data on fish growth performance were statistically analyzed by one-way analysis of variance (ANOVA), using Student-Newman- Keuls test for individual comparisons ($p<0.05$ level of significance). Non- parametric Kruskal Wallis test was used for comparison of water quality parameters in fish tanks since these data were not normally distributed. Student-Newman-Keuls test was used to find the significantly different treatment groups. All statistical analyses were carried out using the SPSS version 17.

Results

Proximate protein composition of medicinal plant ingredients and other feed ingredients used to prepare test diets and control diet are in Table 1. Among medicinal plant ingredients, *S. grandiflora* recorded the highest protein content and *A. marmelos* recorded lowest protein content.

Table 1: Proximate Protein Composition of Medicinal Plant Ingredients and Other Feed Ingredients.

Ingredients	Protein Composition (% Dry Weight)
S. Grandiflora	29.71
A. Marmelos	17.58
A. Sativum	17.95
Fish Meal	44.01
Soybean	40.30
Coconut Meal	16.66
Wheat Flour	14.19

Of the other ingredients, fishmeal and wheat flower recorded the highest and the lowest protein content, respectively. Compositions of control and test diets are in Tables 2 and 3, respectively.

Table 2: Composition of the Control Diet Prepared for the Experiment.

Ingredients	Amounts (g) per 500g of Diet
Fish Meal	201.79
Soya Bean	201.79
Coconut Meal	17.74
Wheat Flour	17.74
Minerals Mixture	60.94

Table 3: Compositions of Test Diets Prepared for the Experiment

Ingredients	Amounts (g) of Test Diet
Common Diet	325
Test Diets *S. grandiflora* (T_1)	175
A. marmelos (T_2)	175
A. sativum (T_3)	175

Growth Performance

In par with the length increments, the growth rate of experimental fish in all treatments gradually increased with time. The highest growth rate was recorded from T_3 tanks in the fifth week, while lowest growth rate was recorded from T_1 tanks in 4th week. Fish in control tanks and T_3 tanks showed the highest growth rates than other treatments (Table 04).

Results of the ANOVA test for 1st and 2nd sampling weeks indicated that there were no significant differences among different treatments. ANOVA test revealed that mean length increment in T_3 tanks in 5th week was significantly higher than other experimental tanks. Also, computed value (0.920± 0.126 cm) was recorded as the highest mean length increment.

Table 4: Comparison of Length Increments in Treatment Groups in Experimental Tanks.

Time Interval	(Length Increment ± SE)/cm			
	T_1	T_2	T_3	C
1st	$0.311^{a} \pm 0.062$	$0.256^{a} \pm 0.104$	$0.311^{a} \pm 0.064$	$0.402^{b} \pm 0.08$
2nd	$0.291^{a} \pm 0.034$	$0.264^{a} \pm 0.069$	$0.387^{ab} \pm 0.033$	$0.458^{b} \pm 0.048$
3rd	$0.420^{a} \pm 0.054$	$0.289^{a} \pm 0.022$	$0.407^{a} \pm 0.028$	$0.589^{b} \pm 0.046$
4th	$0.247^{a} \pm 0.037$	$0.411^{ab} \pm 0.021$	$0.315^{ab} \pm 0.083$	$0.496^{b} \pm 0.034$
5th	$0.513^{a} \pm 0.073$	$0.602^{a} \pm 0.033$	$0.920^{b} \pm 0.126$	$0.573^{a} \pm 0.066$
6th	$0.427^{a} \pm 0.052$	$0.489^{a} \pm 0.029$	$0.556^{a} \pm 0.078$	$0.773^{b} \pm 0.052$

Note: *Letters next to the each value indicate significantly different groups recorded from Student-Newman- Keuls test. Similar letters indicate non-significant different treatments within the experimental time period.*

According to weight increments, growth rate gradually increased with the time in all experimental treatments. The highest growth rate was recorded from control tanks in 6th week (Table 5), while the lowest growth rate was recorded from T_3 tanks in 2nd week. Control tanks showed the highest growth rate. Among medicinal plant treatments, fish in T_3 tanks indicated the highest growth rate.

Mean weight increments in control tanks were significantly different in 3rd, 4th and 6th weeks. The ANOVA test revealed that T_3 tanks in 5th time interval week was significantly higher than the other experimental tanks. Also, mean weight (0.7503 ± 0.0592 g) in 5th week was the highest weight increment from the experimental diets.

ANOVA test indicated that there was a significant difference ($p<0.05$) in condition factor of fish in the experimental tanks (Figure 1). The significantly highest condition factor was recorded among fish in T_1 tanks (1.7057 ± 0.0852) and control tanks (1.6983 ± 0.0287).

Table 5: Comparison of Weight Increments Among Treatments Groups in Experimental Tanks.

Time Interval	(Mean Length Increment ± SE)/cm			
	T_1	T_2	T_3	C
1st	$0.113^{a} \pm 0.012$	$0.085^{a} \pm 0.005$	$0.172^{b} \pm 0.007$	$0.227^{c} \pm 0.010$
2nd	$0.069^{ab} \pm 0.015$	$0.095^{b} \pm 0.003$	$0.052^{a} \pm 0.001$	$0.138^{c} \pm 0.011$
3rd	$0.179^{a} \pm 0.010$	$0.113^{a} \pm 0.005$	$0.191^{a} \pm 0.005$	$0.331^{b} \pm 0.056$
4th	$0.107^{a} \pm 0.011$	$0.175^{a} \pm 0.004$	$0.188^{a} \pm 0.042$	$0.329^{b} \pm 0.024$
5th	$0.325^{a} \pm 0.040$	$0.393^{a} \pm 0.032$	$0.750^{b} \pm 0.059$	$0.711^{b} \pm 0.035$
6th	$0.361^{a} \pm 0.042$	$0.372^{a} \pm 0.033$	$0.425^{a} \pm 0.058$	$0.945^{b} \pm 0.085$

Note: Letters next to the each value indicate significantly different groups recorded from Student-Newman- Keuls test. Similar letters indicate no-significant difference within the experimental time period.

Condition factors of fish in T_1, T_3 and C (control) tanks were significantly different ($p<0.05$) than that in T_2 tank. Fish fed with *Aegle marmelos* leaves registered lowest condition factor (1.47) while fish fed with *S. grandiflora* leaves exhibited highest condition factor (1.71).

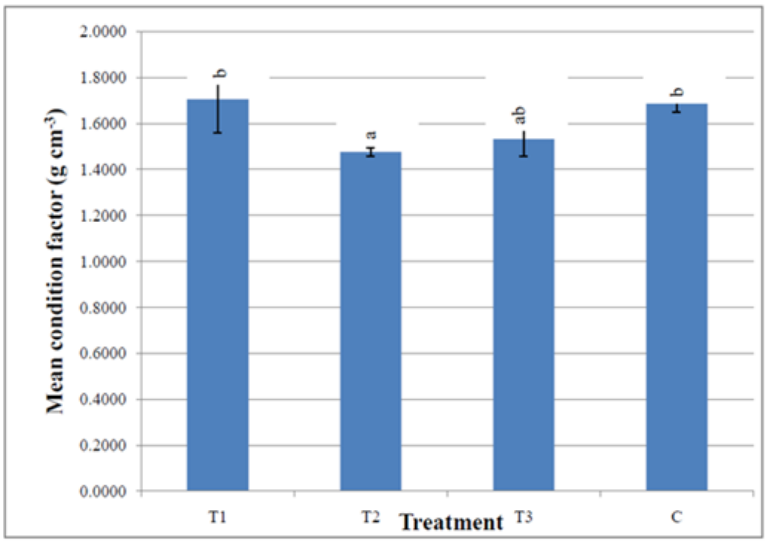

Figure 1: Condition Factors of Fishes in Experimental Tanks

Changes of Water Quality Parameters

There were no significant differences among treatments ($p<0.05$) in water quality parameters except pH. The highest conductivity 114.695±2.4638 (µs/cm), nitrate 0.4043±0.0287 (µg/L), ortho-phosphate 48.952±2.8970 (µg/L) and the lowest mean DO 4.1638±0.4078 (mg/L) were recorded from control tanks. The lowest BOD_5 0.6465±0.0813 (mg/L) was recorded from T_3 tanks (Table 06).

Table 06: Water Quality Parameters in Experimental Tanks.

Parameter	Treatment			
	T_1	**T_2**	**T_3**	**C**
pH*	7.09^a ± 0.07	7.10^a± 0.06	7.11^a ± .08	6.78^b ± .03
Conductivity (µs/cm)	110.51 ±0 .12	111.08 ± 2.15	107.53 ± 1.88	114.69 ± 2.46
Temperature (^oC)	28.02 ± 0.11	27.99 ± 0.12	28.06± 0.11	27.98 ± 0.12
DO (mg/L)	4.62± 0.37	4.84 ± 0.39	4.84 ± 0.42	4.16 ± 0.40
BOD_5 (mg/L)	0.69± 0.06	0.73± 0.10	0.64± 0.08	0.79± 0.08
Nitrate (µg/L)	0.39± 0.03	0.38 ± 0.03	0.37± 0.03	0.40 ± 0.02
Ortho phosphate (µg/L)	46.38± 2.82	46.19 ± 3.20	43.23± 2.78	48.95 ± 0.89

** Indicates significantly different water quality parameters among different treatments at $p < 0.05$. Similar letters indicate no significantly different among food categories within the fish species.*

Discussion

The study has mainly focused on the effect of diets containing medicinal plant ingredients (*Sesbania grandiflora*, *Aegle marmelos* and *Allium sativum*), on the growth performance of red tilapia cultured in indoor fiberglass tanks. Also, water quality parameters were assessed to determine if there is any effect on water quality due to medicinal plant ingredients containing fish diets.

A higher condition factor means that the fish has attained a better condition. Condition factor of fish can be affected by a number of factors such as stress, sex,

season, availability of feeds, and other water quality parameters (Khallaf et al., 2003).

The highest condition factor recorded in the fish in T_1 treatment and control tanks indicates that both *Sesbania grandiflora* containing diet and control diet have provided better environments and nutrients for the growth of fish than T_2 and T_3 treated tanks.

The highest mean weight and length increments in fish were recorded from the fish in control tanks. Of the three treatments, the highest mean weight and length increments were recorded from the fish fed with *A. sativum* diets. The highest mean weight and length increments were recorded from garlic-treated fish at 10^{th} week during the experimental period.

Shalaby et al. (2006) have reported a significant increase in weight gain, feed efficiency, Protein Efficiency Ratio (PER) and Specific Growth Rate (SGR) in Nile tilapia which were fed with a diet containing garlic powder of 30.0 g/kg in diet. Similarly, Diab et al. (2002) have mentioned feeding a diet with 2.5% garlic/kg diet results in the highest growth performance in *O. niloticus*. Also, Abou-Zeid (2002) has found a positive improvement in biomass and specific growth rate in *O. niloticus* with garlic supplementation. Metwally (2009) also found that the best performance was obtained in Nile tilapia fed with garlic powder in 32 g/kg diet.

Hossain et al. (2002) carried out a feeding trial in a warm water recirculation system to evaluate the nutritive value of *Sesbania aculeata* seed meal as a possible fish meal substitute for the diet of tilapia. *Sesbania* seed meal was included in diets at various levels and-fish fed diets containing higher levels (>9.7%) of *Sesbania* meal had shown significantly higher whole-body moisture, lower lipid and gross energy content. The lower growth performance of fish-fed diets containing higher levels of *Sesbania* meal is thought to result from the presence of tannins, saponin and the non-starch polysaccharide content in the seeds.

There may be an unknown factor influencing the growth of fish such as initial weight which may have an essential vigor for growth performance. In this experiment, initial weight of fish in control tanks was higher than in other experimental tanks. So, it may be the vigor for growth performance of fish in control tanks. But, mean relative growth rates in fish tested in both T_3 treated and control tanks have been obtained higher growth rates than other treatments. So, initial length and weight do not affect the fish growth in control tanks. Present observation indicates that small fish are more palatable for a control diet. And it may be the other reason for higher growth in fish in the control tanks. Also, these fish highly prefer garlic-containing fish diet to other medicinal plant ingredients-containing fish diets.

Comparison of mean values of water quality parameters indicates that only pH is significantly different in experimental tanks. Water quality in treatment tanks has not been changed by feeds with medicinal plants through the experimentation. It elucidate that medicinal plant feeds do not alter water quality, and they can be given to fish with feed without harming to the water quality. In the present experiment, same pellet sizes were used, and study found that the pellet size do not affect the water quality. Fish in control and garlic-treated tanks have obtained higher growth rates than others. The palatability of feed by fish may affect for this results. The study confirms that medicinal plant feeds can be given to fish without harming to quality of the water.

Conclusions

The present study concludes that fish in control and garlic treated tanks shows higher growth rates than other fish in the experiment. Small fish are more palatable for both control and garlic-containing fish diets and affect fish growth in the experiment and, medicinal plant feeds can be given to fish without harming to the water quality. Study recommends that the experiment should be continued for a longer period to observe the any changes in water quality parameters and the growth

of fish. Further investigations with a large fish which may be performed higher growth than small fish are recommended to validate the present results.

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