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Growth performance and haematological responses of supplemented feed on *Tectaria zeylanica* (Houtt.) Sledge leaf extract on Giant freshwater prawn *Macrobrachium rosenbergii* (de Man, 1879)

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Abstract

The aim of the present study was carried out to investigate the basal diet (Control) incorporated with ethanolic extract of *Tectaria zeylanica* (Houtt.) (TZE) fed with growth performance and haemocyte parameters analysis of *Macrobrachium rosenbergii* (de Man, 1879*)* for experimental period of 90 days of laboratory methods. The experiments were carried out at the Research Unit of Gayathri Research Foundation, Palayamkottai, during the periods of June 2014 to August 2014. Experimental diets were prepared for control diet incorporated with different concentration of ethanlic extracts of *T. zeylanica* leaves. The growth parameters of weight gain, SGR, and FCR were analyzed. Haematological parameters of THC and DHC were observed. The results of fresh water prawn of *M. rosenbergii* fed with different concentration of all the ethanolic extract of *T. zeylanica* was showed very significant growth responses of weight gain, SGR, and FCR. Maximum growth responses of 4 % of ethanolic extract *Tectaria zeylanica* were observed. All the concentration of ethanolic extract incorporated fed *M. rosenbergii* was observed for continuously increased for THC and DHC in experimental periods. Differential haemocytes (DHC) of *M. rosenbergii* fed with extract of *T. zeilanica* (Holtt.) was observed by three types of hemocytes in agranular (hyaline), small-granule, and large-granule cells. In conclusion of the present study suggested that *T. zeylanica* (Houtt) Sledge can be used as enhance the growth parameters in *M. rosenbergii*.

Key words: Crustacean: *Macrobrachium rosenbergii*. *Tectaria zeylanica,growth,* haemocytes

Introduction

Aquaculture is one of the big opportunity industry for developing countries in the world food market (Manoj and Vasudevan, 2009; Ahmed *et al*., 2013; Hossain *et al*., 2013). FAO,(2009) reported that 50 million tonnes of annual production of fish and shellfish in aquaculture industry.

The genus *Macrobrachium* is well known because of the number of species, wide geographic distribution, and commercial importance (Holthuis, 1952; Villalobos, 1982). *Macrobrachium* species was produced in the five major countries of China (56.3% of 2007 global production) followed the Thailand (12.5%), India (12.3%), Bangladesh 11% and Taiwan (4.5%). The total global production of *Macrobrachium* species was estimated that 7,50,000 - 10,00,000 tonnes per year (New, 2005). *Macrobrachium rosenbergii* is an important species are cultured in many countries. It is locally known as attu eral in tamil name, it is the biggest freshwater prawn in the world, the male maximum growing size of 320 mm and weighing over 200gms in size.

Plant products have been reported to promoted the various activities like anti-stress, growth promotion, appetite stimulation, tonic, immunostimulation and to have antimicrobial properties in finfish and shrimp (Hiam El-Desouky *et al*.,2012). The development of feed formula is a major input in the hatchery of aquaculture industry, the availability of low cost-effective feeds play an important role in aquaculture industry (Chunchom *et al*., 2010). Feed additives are very useful to improve the growth enhance the fish and prawn. Several feed additives are incorporated with plants and plants to substitute or minimize quantity of used chemicals through the global trend to go back to the nature. The developing countries used for derive a substantial part of their subsistence and income from wild plant products. Wild plants are provided a staple food for indigenous people, serve as complementary food for non-indigenous people and offer an alternative source of cash income (Uprety *et al*.,2012).

Tectaria zeylanica (Houtt.) Sledge is belonging to the family Dryopteridaceae. Leaves were used for vegetable (Uprety *et al*.,2012). Earlier studies, phytochemical analysis of plants part was screened for the active constituents of alkaloids and flavonoids were more abundant in the leaves of *Tectaria zeilanica* (Maridass and Racichandran,2009). The main objectives of the present study, to evaluation of the growth performance and hematological analysis of *M. rosenbergii*

fed on supplementary diets on extract of *Tectaria zeilanica* leaves.

Fig.1: Natural Habit of *Tectaria zeilanica*

2. Materials and Methods

2.1 Collection of Plant Materials

Fresh leaves of *Tectaria zeilanica* (Holtt.) Sledge were collected from Upper Kothaiyar, and Periyamylor in Kalakad-Mundanthurai Tiger Reserve (KMTR), Southern Western Ghats, Tirunelveli District, Tamil Nadu. A voucher specimen was deposited in the Centre for Biodiversity and Biotechnology, St. Xavier's College (Autonomous), Palayamkottai, Tamil Nadu, India.

2.2 Extraction

1kg of powdered materials of *Tectaria zeilanica* (Holtt.) Sledge leaves were separately Soxhlet extracted with ethanol for 5hrs. The solvent was removed under reduced pressure.

2.3 Experimental Diets

The control and experimental diets were prepared the composition mention in the table-1.

Table -1: Feed preparation and composition mixture of control diet and experimental diets

Ingredients	Composition (%)	Experimental Diets			
		1%	2%	3%	4%
Groundnut oil cake	45	45	45	45	45
Soybean meal	18	18	18	18	18
Fish meal	17	17	17	17	17
Rice bran	17	16	15	14	13
Mineral and vitamin mixa	2.8	2.8	2.8	2.8	2.8
Carboxy methyl cellulose	0.2	0.2	0.2	0.2	0.2

a Each 250 g vitamin and mineral mixture provides vitamin A (5,000,000 IU), vitamin D3 (100,000 IU), vitamin B2 (0.2 g), vitamin E (75 units), vitamin K (0.1 g), calcium pantothenate (0.25 g), nicotinamide (1.0 g), vitamin B12 (0.5 mg), choline chloride (15 g), calcium (70 g), manganese (2.75 g), iodine (0.1 g), iron (0.70 g), zinc (1.5 g), copper (0.2 g) and cobalt (0.05 g).

2.4 Experimental design

2.4.1 Feeding and acclimatization of laboratory conditions

The experimental animals of *M. rosenbergii* were collected from Tamiraparani River, Attur, near sea shore area of Tuticorin district, Tamil Nadu. They were transported to the laboratory in polythene bags filled with oxygenated water and maintained in a cement tank for one month. They were fed ad libitum with boiled egg albumin twice a day and *Artemia* nauplii once a day alternatively at 10% of body weight. On daily basis three fourth of the water was renewed by siphoning method causing minimum disturbance to the prawns. The unfed feed if any and the excreta were removed.

2.4.2 Experimental Setup

2.145gms of experimental animals of *M. rosenbergii* were using for the present study. The experimental animals were selected for five group and triplicate method. Each group 10 number of prawn were selected and maintained in the 50lr trough and acclimatize for the one week. Initial study, each group of 10 prawn were selected and taken for the measurements of length and weight. Experimental and control feed were prepared by basal diet and basal diet incorporated with different concentration of ethanolic extract on *T. zeilanica* (Table-1). Prawns were daily fed with two times per day at about 8:00 and 16:00hr at 15% of their body weight for 90days. Uneaten feed and faecal matter were siphoned out before the first feeding in the morning. The amount of feed given was adjusted according to these weight measurements of prawns. The experimental animals were weighed in groups of 10 on a digital weighing machine and the mean weights were determined and they were returned to their same tanks. At the end of the experiment, the survival, mean individual weight and total length of the prawn were determined. Growth parameters were calculated as follows: final body weight (WG) = final body weight (g) – initial body weight (g). Specific growth rate (SGR) (%BW day-1) = (Ln final body weight (g) - Ln initial weight (g))/ (experimental period)×100. Feed conversion ratio (FCR) (%) = (total fed/body weight increase (g))×100.

2.4.5 Total and differential hemocyte counts

The end of the experimental periods, the collection of haemocyte in *M. rosenbergii* fed with control and experimental diet. The total hemocyte of *M. rosenbergii* were counted in the Neubauer hemocytometer chamber. The

differential counts of *M. rosenbergii* were performed on slides prepared with 100µl of a diluted cell suspension ($3x10^6$cells). The hemocyte monolayer was fixed with 1 % glutaraldehyde in 3.2 % NaCl for 30 min at 4 °C. The hemocytes were stained with a May-Grünwald solution (3min) followed by a Giemsa solution (1:10 dilution for 10 min), and dehydrated with ethanol. After immersion in xylene (6 min), the slides were closed with a Eukitt mounting medium (Fluka) (Celi *et al*., 2013). The cells were then counted in random areas, and the numbers and relative proportions of hemocyte types were calculated by counting at least 200 cells on each slide. The cells were observed under a microscope, and the DHC was determined using the following equation:

THC = Number of counted haemocytes in the 4 corners ×2×¼×10 ×the dilution factor.

$$\text{DHC (\%)} = \frac{\text{Number of different hemocyte cell types}}{\text{Total hemocyte cells counted}} \times 100$$

2.4.6 Identification of Haemocytes

The collection of haemocyte of *M. rosenbergii* fed with control and experimental diet (incorporated with ethanalic extract) observed for initial and final day of experimental periods. Differential haemocyte counts (DHC) are determined the using smearing and staining techniques. The haemocytes type of *M. rosenbergii* were identified by differentiated between hyaline (agranular) and granular cells (granulocytes and semi-granulocytes) using differences in size, morphology and granular content (Le Moullac *et al*., 1997). The identification of haemocytes good method to recognize and differentiate granulocytes, agranulocytes and semi-granulocytes, hyaline cell (agranulocyte), small granular cell and large granular cell in the blood smear (Stolen *et al*., 1995; Kondo, 2003; Kondo, 2003).

2.5 Statistical analysis

Data for all measured parameters were analyzed using SPSS for windows, Version11.0. The variations from dietary treatment were compared by one-way ANOVA. The Tukey HSD post hoc analysis was used to detect differences between means. Significant differences were considered at <0.05. All percentage values were normalized through a square root arcsine transformation before statistical treatment.

3. Results and Discussion

3.1 Growth performance

The results of growth performance of experimental diet incorporated with different concentration of *Tectaria zeilanca* leaf extract fed with *M. rosenbergii* was observed for 90 days represented in the table-2. All the concentration of control diets incorporated with experimental diets have been observed by gradual increasing in the growth performance parameters in weight gain, and total length. The percentage of survival rate and specific growth rate (SCR) of *M. rosenbergii* were observed by no mortality was observed by all the experimental diets. The maximum weight gain (%) of *M. rosenbergii* was observed by 4% of extract of *T. zeilanca* incorporated with control diet. The maximum weight gain values 36.31gms was observed by 4% control diet incorporated with *T. zeilanca* leaves extract. The mean values of SGR and FCR values were seen in the table-2. *M. rosenbergii* fed the control diet had the lowest survival rate of 70% was observed. The overall experiment results fed with *Tectaria zeilanca* (TZE) extract incorporated with control diets exhibited in the good improvement of body weight, weight gain, feed efficiency of *M. rosenbergii*. The fresh water prawns of *M. rosenbergii* fed with control diet containing ethanol extract at 4% concentration observed by significant number of moults was observed.

3.2 Total Haemocytes

Table -2 shows that the ethanolic extract of *T. zeilanca* (TZE) incorporated with control diet enhances with growth parameters and good responses of increasing number of total haemocytes (THC) and differential haemocytes (DHC) were identified in *M. rosenbergii*. Total hemocyte counts (THC) was significantly increased in *M. rosenbergii* fed with different concentration of TZE supplementation feed for experimental period. However, the THC was significantly increased in prawn *M. rosenbergii* fed with all experimental diets when compared to the control diet (Table-2). In the present study, increasing number of haemocyte counts was very useful for the indicator of prawn health. The total haemocytes counts (THC) was $23x10^6$ cell/ml observed by *M. rosenbergii*. The highest percentage of granulocytes and semigranulocytes were observed in all experimental diet (Table-2). The lowest percentage of hyalinocytes were observed in all experimental diets compared than the control diet. Earlier study, hyaline cells of *M. rosenbergii*, posses 70% of hemocytes (Vazquez *et al*., 1997), in contrast to Penaeidae shrimp. In the lobster, *Homarus americanus,* and the crab, *Loxorhynchus grandis*, the SGH were highest (similar to *F. indicus)* reaching more than 60% of the total cell number (Hose *et al*., 1990). The total haemocyte counts is a useful indicator of prawn health (Sarathi,2007). Earlier study, haemocytes of crustacean species maximum number of haemocytes influence the moulting development, reproductive status, nutrition conditions, and cure of diseases (Le Moullac *et al*.,1997; Cheng and Chen,2001). According to Martin and Graves (2005) reported that low level of THC (about $11×10^3$ cells/mm^3) in the *Penaeus californiensis*. Earlier studies on Tsing *et al*. (1989) reported about $5\text{-}14×10^6$ cells/mm^3 in the hemolymph of the both species of *Penaeus japonicas* and *F. indicus*. Yeager and Tauber (1935) observed that THC recorded $8.9×10^6$ in *Penaeus setiferus.*

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Table-2: Growth performance of *Macrobrachium rosenbergii* fed with or without TZE for 90 days

Concentrations	Initial day		Final day		Length gain (cm)	Wait gain (gms)	Survival Rate (%)	SCR (%)	FCR (%)	Total Haemocytes	Differential Haemocytes (%)		
	Mean Length (cm)	Mean Weight (gms)	Mean Length (cm)	Mean Weight (gms)							Hyalinocyte	granulocyte	Semi-granulocyte
Control (Basal diet)	3.75±0.16	2.145±0.16	10.34±0.16	28.67±0.16	6.6±0.16	26.52±0.16	100	2.38	1.98	$26.3x\ 10^6$	46	7	47
Basal diet with 1 % of AE	3.75±0.16	2.145±0.16	10.89±0.16	31.34±0.16	7.14±0.16	29.20±0.16	100	2.45	1.73	$28.1x\ 10^6$	43	9	48
Basal diet with 2 % of AE	3.75±0.16	2.145±0.16	11.34±0.16	34.23±0.16	7.59±0.16	32.42±0.16	100	2.69	1.56	$45.6x\ 10^6$	42	8	50
Basal diet with 3 % of AE	3.75±0.16	2.145±0.16	13.56±0.16	36.56±0.16	9.81±0.16	34.42±0.16	100	2.98	1.25	$51.7x\ 10^6$	37	15	48
Basal diet with 4 % of AE	3.75±0.16	2.145±0.16	14.98±0.16	38.45±0.16	11.23±0.16	36.31±0.16	100	3.21	0.98	$52.1x\ 10^6$	37	14	49

3.3 Differential Haemocytes Counts

In the present study, basal diets incorporated with *T. zeilanca* (TZE) extract fed on *M. rosenbergii* was observed by three type of cell in hyalinocytes, granulocytes and semi-granulocytes. These haemocytes were easily identified by different numbers of granules size present in the cytoplasm; hyaline cells (HC) with round, oval or elongate shaped, no granule and pink or light red cytoplasm, small granular cells (SGC) with oval shaped, small granules and light red cytoplasm and large granular cells (LGC) with round or oval shaped and large granules. Identification of haemocytes were based on the presence of cytoplasmic granules present in the cells were identified by light or electron microscopy. Small granulocytes were responsible for the phagocytosis of foreign particles (Hose and Martin, 1989). Crustacean species of haemocytes are the main actors in the immune responses (Johansson *et al*., 2000; Irving *et al*., 2005; Rodrigues *et al*., 2010). The DHC have provided varying results between crustacean species. In the present study, we observed haemocytes of hyalinocytes and semigranulocytes were more represented in the circulating hemolymph of *M. rosenbergii*. Haemocytes was involved in the immune response system, in which each as a distinct function. Haemocytes of Hyalinocytes (5- 15% of circulating hemocytes or CE) are small nonrefractive cells, with a small nucleus relative to their cytoplasm, which have few or no cytoplasmic granules. The primary role of these cells is related to clotting and phagocytosis (Zhang *et al*., 2006). Granulocytes (10-20% of CE) have the smallest nucleus and a high number of cytoplasmic granules (0.8µm width). Granulocytes display phagocytic activity and store the enzyme prophenoloxidase (proPo). These cells may be stimulated by β-1,3-glucans, peptidoglycans (PG) and lipopolysaccharides (LPS) to provoke exocytosis and enzyme release. Their function is encapsulation, initiating the proPO cascade and phagocytosis (Zhang *et al*., 2006). Semi-granulocytes (75% CE) have a large number of small granules (0.4µm width) similar to vertebrate granulocytes. These cells possess β-1,3-glucans receptors and their principal function involves phagocytosis, encapsulation and clotting (Martin and Graves, 2005; Zhang *et al*., 2006). Differential hemocyte count values were recorded in the relative percentage (differential hemocyte count) of the hemocytes in the different species. Hose *et al.,* (1992) reported that the HC comprises 50–60% of the circulating hemocytes of In *Sicyonia ingentis*, whereas the SGH and LGH represented 30% and 10%, respectively. The results of the present study indicated that the growth performance of a prawn *M. rosenbergii* experimental diet fed on significant results compared than control diet. Earlier review on various extracts from herbs and spices are reported to improve animal performance by stimulating action on gut secretions or by having a direct bactericidal effect on gut microflora and furthermore the herbals active principles in the diets induce the secretion of the digestive enzyme and the growth promoter in herbs induced high protein synthesis (Citarasu,2010). Herbal products are effectively useful in preparation of herbal maturation diet (Citarasu *et al*., 2003, Immanuel *et al*., 2003, Michael Babu *et al*., 2001, Michael Babu *et al*., 2009). The DHC have provided varying results between crustacean species. Both haemocytes of hyalinocytes and semigranulocytes were more represented in the circulating hemolymph of *C. borealis* and *C. pagurus*. Vázquez *et al*., (1997) reported that hyalinocytes comprised 70 % of the total hemocytes and no semigranulocytes were found in *M. rosenbergii*. In *Sicyonia ingentis*, 50 - 60 % of the circulating hemocytes were hyalinocytes, whereas the semigranulocytes and granulocytes comprised 30 % and 10 % of the total, respectively (Hose and Martin, 1989). High percentages of hyalinocytes (five to eight times more abundant than granulocytes) have been observed in the crab *Eriocheir sinensis* (Bauchau and Plaquet, 1973), the lobster *Panulirus interruptus* (about 56 %) (Hose *et al*., 1990) and the crayfish *Procambarus clarkii* (more than 70 %) (Lanz *et al*., 1993). Herbs, rich sources of immune enhancing substances, are used to promote health, increase the body's natural resistance to infection and in prevention and treatment of various diseases (Devasagayam and Sainis, 2002). The

conclusion of the present study experimental diets of TZE was good results of growth and immunomodulation activity was observed. The hemocytes play a central role in the immune responses of defenses of crustaceans, the total and differential hemocyte counts provide a useful way of assessing the physiological state of an animal. The observed results suggest that dietary TEZ extract for *M. rosenbergii* could positively affect growth performance, feed utilization and immunomodulation activity.

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