Effects of *Piper longum*, *Piper nigrum* and *Zingiber officinale* on survival, growth, activities of digestive enzymes and contents of total protein, vitamins and minerals in the freshwater prawn *Macrobrachium rosenbergii*

P. Saravana Bhavan*, C. Saranya, N. Manickam, T. Muralisankar, S. Radhakrishnan and V. Srinivasan
Department of Zoology, Bharathiar University, Coimbatore – 641046, Tamilnadu, India.

**ABSTRACT**
In this study, *Piper longum* (long pepper), *Piper nigrum* (black pepper) and *Zingiber officinale* (dried zinger) were incorporated with artificial feeds formulated to understand their effects on survival, growth, activities of digestive enzymes and biochemical constituents in *Macrobrachium rosenbergii* post larvae (PL). These herbs were independently incorporated with basal ingredients at a concentration of 5% each, and fed to *M. rosenbergii* PL (1.56±0.08 cm; 0.074±0.02 g) for a period of 60 days under laboratory condition with triplicates. A significant improvement (P<0.05) in survival and growth performance (weight gain, specific growth rate, food conversion ratio), elevation in activities of digestive enzymes (protease, amylase and lipase), increased concentrations of total protein, non-enzymatic antioxidants (vitamins C and E), and mineral salts (sodium and potassium) were recorded in *P. longum* incorporated feed fed PL followed by *P. nigrum* and *Z. officinale* when compared with control. Polypeptide bands of molecular weight between 116-14 kDa were resolved in the muscle of PL fed with control as well as herbs incorporated feeds. Generally, there were eleven Coomassie blue stained protein bands (116, 58, 51, 45, 38, 33, 25, 18, 17, 16 and 14kDa) were calculated in herbal incorporated test samples. The general comparison between control and experimental groups revealed that there was more number of bands resolved in herals incorporated feeds fed PL. Therefore, it is suggested that these medicinal herbs have their own influence in protein synthesis. In this study, the overall results indicated the fact that these herbs have acted as appetizers and hence, enhanced the secretion of digestive enzymes, which facilitated efficient digestion, absorption of nutrients and favoured for general health, which in turn ultimately produced better survival and growth of *M. rosenbergii* PL. Therefore, these herbs can be taken as feed additives in sustainable development of freshwater prawn culture.

© 2013 Elixir All rights reserved.

**Introduction**
The giant river prawn, *Macrobrachium rosenbergii* is dominating in India, and it is one of the major contributors of national economy. It is good source for protein, essential amino acids and polyunsaturated fatty acids, it is very low in fat, and therefore, it can be used as a healthy choice of food for human (Bhavan et al. 2010). The successful prawn culture depends on quality live feeds and nutrient rich commercial feeds. Both are very costly and non-affordable to small farmers. Therefore, low cost artificial feeds are much needed for promoting inland aquaculture of freshwater prawns.

Nowadays, herals are widely used in veterinary and human medicine. The herbal active principles in the diets induce the secretion of the digestive enzymes. It would stimulate the appetite and ultimately increased the food consumption and efficiencies (Bhavan et al. 2011; Shanthi et al. 2012; Radhakrishnan et al. 2013). Herbal active principles induce transcription, which lead to high protein synthesis (Citrasu et al. 2002, 2009). Several plant products found to have potent antiviral activity against fish and shrimp viruses. For example, shrimp fed with ethanolic extract of *Clinacanthus nutans* showed 95% survival rate against yellow head virus (YHV), but the control showed only 25% survival (Direkbusarakom et al. 1996). Herbs such as, *Solanum trilobatum*, *Psoralea corylifolia* and *Clematis paniculata* enriched Artemia fed *Peneaus monodon* had reduced vibrio species (Citrasu et al. 2002, 2009). Many plants have antibacterial and antifungal properties. For instance, the extract of *Ocimum basilicum* has controlled infection of *Aspergillus flavus* and *Fusarium oxyspoum* (Adiguzel et al., 2005). Herbal compounds have the ability to inhibit the generation of oxygen anions and scavengen free radicals, hence reduces the effects due to stress.

*Piper longum* (long pepper) is a component of medicines which is reported as good remedy for treating gonorrhea, menstrual pain, tuberculosis, sleeping problems, respiratory tract infections, chronic gut-related pain and arthritic conditions. It has antioxidant, antimicrobial, antiinflammatory, antiallergic, immunomodulatory and antitumour activities (Joy et al. 2010; Khushbu, 2011). Other beneficial effects of *P. longum* include analgesic and diuretic effects, relaxation of muscles tension and alleviation of anxiety. Alkaloids, pipeine, piper longumine and pipernonaline are the active principles of *P. longum*.

*Piper nigrum* (black pepper) has many medicinal uses, including the ability to control worm infestations, and to provide...
relief for a number of ailments including asthma, cough, heart
diseases, throat inflammations, night blindness, urinary
disorders, tooth and muscle aches, inflammations, snake bites,
eye diseases, cholera, and swoons. It has antibacterial,
antifungal, antioxidant, antihypertensive, antimalarial and
antileukemic activities (Pundir et al. 2010; Bai et al. 2011).
The roots of pepper also have medicinal qualities, as a stomach
anesthetic, analgesic, muscle relaxant, digestive stimulant,
antiseptic, diuretic, sudorific (diaphoretic, promotion of
sweating), anxiolytic, and as a hypotonic (Nelson & Eger, 2010).
Externally it is valued for its rubefacient properties and as a local
application for relaxed sore throat, piles and some skin diseases
(Pullaiah, 2002). In aquaculture, P. nigrum plant extracts posses
antibacterial activity against Edwardsiella agents and bacteria
tested in ornamental sea anemone, Radisanthus ritteri (Wei et al.
2007). Piperine and piperoyl-piperidine are two major herbal
principles. Piper extracts and piperine possess inhibitory
activities on prostaglandin (Misha, 2010). It is also used as a
purgative, to induce vomiting and scorpion-sting (Sumy et al.
2000).

Zingiber officinale (dried ginger) is used as ayurvedic
medicine to cure indigestion, stomachache, abdominal pain,
disorders of gallbladder, hyperacidity, hypercholesterolemia,
hyperglycemia, morning sickness, prevention of motion
sickness, and nausea (Yohannes, 2010). It possesses
antimicrobial, antipyretic, ectoparasitic, antioxidant, antinflammatory
and immunomodulatory activities (Yusof et al. 2002; Sasisdharan
et al. 2010; Famurewa et al. 2011). It also used as an antietemic
agent (Phillips et al. 1993). In aquaculture, the rainbow trout
(Oncorhynchus mykiss) fed with 1% ginger extract exhibited
increased phagocytic activity of blood leukocytes (Chung &
Secomes, 1987). Its active principles are gingerol, zingerone
and shogaol.

The present study was aimed to understand the influence of
incorporation of medicinal herbs, P. longum, P. nigrum and Z.
officinale at a concentration of 5% each with basal ingredients
on the survival rate growth performance, activities of digestive
enzymes, and the basic biochemical constituents, such as total
protein, vitamins (C and E), minerals (sodium, Na+, and
Potassium, K+) and protein profiles in M. rosenbergii PL.

Materials and Methods

The post larvae (PL-15) of the freshwater prawn, P. rosenbergii were procured from Aqua Hatcheries, Happy bay
annexe, Mugaiyur Village, ECR, Cheyyur Taluk, Kanchipuram
District, Tamilnadu, India. They were transported to the
laboratory in polythene bags filled with hatchery water (pH,
6.8; total dissolved solids, 1.2 g/L; dissolved oxygen, 6.5 mg/L;
BOD, 42.0 mg/L; COD, 140.0 mg/L; ammonia, 1.20 mg/L) and
acclimatized for two weeks (up to PL-30) using ground water
(pH, 7; total dissolved solids, 1200 mg/L; dissolved oxygen, 7.2
mg/L; BOD, 30.0 mg/L; COD, 125.0 mg/L; ammonia, 0.028
mg/L). During acclimatization, the PLs were fed with boiled egg
albumin and Artemia nauplii alternatively twice a day, and latter
they were only maintained with commercially available scampi
feed. Water was adequately renewed daily. At the same time, the
faecal matter and unfed feed were removed. The medium
was adequately aerated.

Ten different types of iso-caloric diets containing
approximately 40% protein, 33% carbohydrate and 12% lipid
were formulated by using the following basal ingredients chosen
on the basis of their nutritional status and year round availability
in the local market, such as fishmeal (25%), soy meal (25g),
groundnut oil cake (25g), and wheat bran (10g). Tapioca flour
(7g) and egg albumin (5 ml/ 100 g) was added as binding agents.
Before addition of the egg albumin the mix was cooked for 15
minutes at 95-100°C and cooled at room temperature. Sunflower
oil (2 ml) was added as lipid source. Vitamin B-complex with
vitamin-C (1 %) was also mixed. A pinch of salt was also added.
The basal diet has 3271 k.cal/kg of energy. With this, each
herbal powder, P. longum, P. nigrum and Z. officinale was
separately added at a concentration of 5% each and mixed well.
The dough was manually pressed through a locally
manufactured feed pelletizer. The pellets were dried in well-
aerated place under shade for 2 days until they became
sufficiently dried. Finally, the pellets were stored in air tight
containers and kept in a cool, dry place and fed to M. rosenbergii PL.

The experiment was conducted in triplicate with 25 PL each
(PL30-90; 1.56±0.18 cm; 0.074±0.02 g) in 30 plastic aquaria of
25 L capacity (each herb represents three diets with a common
control). Each group was fed with a specific herb incorporated
diet ad libitum (1g per aquarium) for a period of 60 days. The
water medium was renewed daily by siphoning method without
severe disturbance to the PL and aerated. The faecal matter,
unfed feed, and exuvia if any were collected separately. Similar
experimental set-ups were maintained few times as and when
required to study various parameters. The nutritional indices
were calculated from the initial and final morphometric data.

Weight Gain (WG) = Final weight (g) – Initial weight (g)

\[
\text{Total quantity of feed consumed (dry wt.)} = \text{No. of prawns introduced} \times 100
\]

Feed Conversion Ratio (FCR) = \[ \frac{\text{Total quantity of feed consumed (dry wt.)}}{\text{Weight Gain (g)}} \]

Survival Rate (SR) = \[ \frac{\text{No. of live prawns}}{\text{No. of prawns introduced}} \]

Activities of digestive enzymes, such as protease, amylase
and lipase were assayed on final day of feeding trial. The
whole flesh except eye stalk, appendages and exoskeleton was
homogenized in ice cold distilled water and centrifuged at
10,000 rpm under 4°C for 20 minutes. The supernatant was used
as crude enzyme source. Protease activity was estimated by the
method of Furne et al. (2005). One unit of enzyme activity
represents the amount of enzyme required to liberate one µg of
tyrosine per minute under assay conditions. Amylase activity
was assayed followed by the method of Bernfeld (1955) in
which the increase in reducing power of buffered starch solution
was measured. The specific activity of amylase was calculated
as mg of maltose liberated/ g of protein/ h (mg/g/h). Lipase
activity was assayed by the method of Furne et al. (2005). The
amount of free fatty acid released per unit time was estimated by
the amount of NaOH required to maintain pH constant and
represented as mille equivalents of alkali consumed.

The concentration of total protein in the muscle was
determined by the method of Lowry et al. (1951) using alcohol
precipitated sample. For estimation of soluble protein, the
muscle tissue samples were first defrosted in phosphate buffer,
PH 7.4 (137 mM NaCl, 2.7 mM KCl, 10 mM Na2HPO4 and 2
mM KH2PO4), homogenized under ice cooled condition and
centrifuged at 1500 rpm at 4°C for 5 min. The soluble protein
content in supernatant was determined by following the method
of Lowry et al. (1951). SDS-PAGE was performed on vertical
slab gel with 4% stacking and 10% separating gels (Laemmli,
1970). Samples of control and the best concentration of each
herb incorporated feed fed PL were taken into consideration.
Protein marker consisted of six different molecular weights (Medox-Bio Pvt. Ltd., India), such as β-galactosidase (116 kDa), bovine serum albumin (66 kDa), ovalbumin (45 kDa), carboxylic anhydrase (29 kDa), soya bean trypsin inhibitor (20 kDa) and lysozyme (14 kDa) was also run to calculate the kDa of various bands resolved in unknown sample. The banding pattern between control and test was compared by information on apparent molecular masses and intensity.

Concentrations of vitamin-C (ascorbic acid) and vitamin-E (α-tocopherol) were estimated in the muscle of PL following the method of Roe & Kuether (1943) and Baker et al. (1980) respectively. Contents of minerals, Na' and K' were estimated in the muscle of PL following the simple flame photometric method of Jeffery et al. (1989) by using Elico CL 220 flame photometer. The values are calculated by adopting the following formula:

\[
\text{Sample reading} \times \frac{\text{Standard reading}}{\text{Sample concentration}} \times \text{Concentration} \times \frac{\text{Purity of NaCl/ K}}{\text{Content (mg)}}
\]

The data were subjected to statistical analyses by adopting ‘two-tailed paired sample t-test’ through SPSS software (version 16) and the significance was noted.

Results and Discussion

Morphometry and nutritional indices

The length, weight, weight gain, survival rate and specific growth (SGR) rate were found to be significantly increased (P<0.05) in experimental feeds fed PL groups when compared with control. The reverse trend was seen in feed conversion ratio (FCR), which indicates the quality of the experimental feeds. Among the three herbs used, \( \text{P. longum} \) incorporated feed fed PL showed the best performance followed by \( \text{P. nigrum} \) and \( \text{Z. officinale} \) (Table 1). The increase in growth performance has also previously been reported in \( \text{M. rosenbergii} \) fed with \( \text{Ocimum sanctum} \), and \( \text{Withania somnifera} \) incorporated feeds (Bhavan et al., 2011), \( \text{Andrographis paniculata} \), \( \text{Cissus quadrangularis} \), and \( \text{Eclipta alba} \) incorporated feeds (Shanthi et al., 2012) and greens, \( \text{Muraya koenigii} \), \( \text{Coriandrum sativum} \) and \( \text{Menthe arvensis} \) incorporated feeds (Bhavan et al., 2012).

Digestive enzymes

The activities of digestive enzymes, such as protease, amylase and lipase were found to be significantly increased (P<0.05) in experimental feeds fed PL groups when compared with control. Among the three herbs used, \( \text{P. longum} \) incorporated feed fed PL showed the best performance followed by \( \text{P. nigrum} \) and \( \text{Z. officinale} \) (Table 1). Increase in activities of protease, amylase and lipase has also previously been reported in \( \text{M. rosenbergii} \) PL fed with \( \text{A. paniculata} \), \( \text{C. quadrangularis} \), \( \text{E. alba} \), \( \text{M. koenigii} \), \( \text{S. sativum} \) and \( \text{M. arvensis} \) incorporated feeds (Shanthi et al., 2012; Bhavan et al., 2012). The increased activities of digestive enzymes reflect the fact that the appetite was induced, which in turn influenced food consumption, and facilitated effective digestion, absorption and ultimately growth of PL.

Total protein

Concentration of total protein was found to be significantly increased (P<0.05) in experimental feeds fed PL groups when compared with control. Among the three herbs used, \( \text{P. longum} \) incorporated feed fed PL showed the best performance followed by \( \text{P. nigrum} \) and \( \text{Z. officinale} \) (Table 1). Increase in total protein has also previously been reported in \( \text{M. rosenbergii} \) PL fed with \( \text{O. sanctum} \), \( \text{W. somnifera} \), \( \text{A. paniculata} \), \( \text{C. quadrangularis} \), \( \text{E. alba} \), \( \text{M. koenigii} \), \( \text{S. sativum} \) and \( \text{M. arvensis} \) incorporated feeds (Bhavan et al., 2011, 2012; Shanthi et al., 2012).

Protein Profile

Polypeptide bands of molecular weight between 116-14 kDa were resolved in the muscle tissue of PL fed with \( \text{P. longum} \), \( \text{P. nigrum} \) and \( \text{Z. officinale} \) incorporated feeds, and control as well (Fig.1). Generally, there were eleven Coomassie blue stained protein bands (116, 58, 51, 45, 38, 33, 25, 18, 17, 16 and 14 kDa) were calculated in herbal incorporated test samples against the standard markers of 116, 66, 45, 29, 20 and 14 kDa, which represent β-galactosidase, Bovine serum albumin, ovalbumin, carboxylic anhydrase, soyabeans trypsin inhibitor and lysozyme respectively. In \( \text{P. longum} \) incorporated feed fed PL all these bands were stained more intensely than \( \text{P. nigrum} \) and \( \text{Z. officinale} \) incorporated feeds fed PL. Major changes in experimental groups were seen in the region between 33kDa-25kDa, and between 17kDa-14kDa. In the case of control, the 116kDa, 25kDa, 18kDa and 17kDa were completely lack of stained bands. The general comparison between control and experimental groups revealed that there was more number of bands resolved in herals incorporated feeds fed PL. Therefore, it is suggested that these medicinal herbs have their own influence in protein synthesis.

![Figure 1: Protein profile of the muscle tissue of M. rosenbergii fed with P. longum, P. nigrum and Z. officinale incorporated feeds](image)

M, marker proteins; C, control; 1, \( \text{P. longum} \); 2, \( \text{P. nigrum} \); 3, \( \text{Z. officinale} \).

Concentrations of vitamins and minerals

Concentrations of non-enzymatic antioxidants, vitamin-C and vitamin-E, and minerals, Na' and K' were found to be significantly increased (P<0.05) in experimental feeds fed PL groups when compared with control (Table 1). Among the three herbs used, \( \text{P. longum} \) incorporated feed fed PL showed the best performance followed by \( \text{P. nigrum} \) and \( \text{Z. officinale} \) (Table 1). Elevation in concentrations of vitamin-C and vitamin-E has previously been reported in \( \text{M. rosenbergii} \) PL fed with \( \text{A. paniculata} \), \( \text{C. quadrangularis} \), \( \text{E. alba} \), \( \text{M. koenigii} \), \( \text{S. sativum} \) and \( \text{M. arvensis} \) incorporated feeds (Shanthi et al., 2012; Bhavan et al., 2012).
Increase in concentrations of Na⁺ and K⁺ has also previously been reported in *M. rosenbergii* PL fed with *Alternanthera sessilis, E. alba* and *C. quadrangularis* incorporated feeds (Radhakrishnan et al. 2013).

Vitamin C and E (ascorbic acid and α-tocopherol) respectively are potent antioxidants, and scavenger reactive radicals, such as hydroxyl, perhydroxyl, peroxy and nitric oxide (Halliwell & Gutteridge, 2001; Bendich et al. 1986; Karakoe et al. 1997). Vitamin E particularly reduces peroxyl radicals in membrane lipids and prevents lipid peroxidation (Burton & Traborg, 1990).

The macro elements sodium and potassium in animal are connected with the regulation of intracellular osmotic pressure and the maintenance of acid-base balance. Particularly, potassium is required for glycogen and protein synthesis, and the metabolic breakdown of glucose.

It is evident that *P. longum, P. nigrum* and *Z. officinale* have the ability to induce secretion of protease, amylase and lipase in *M. rosenbergii* PL, which in turn associated with active principles of these herbs and minerals suggested enhanced protein synthesis. The elevated levels of vitamin-C and E suggested effective scavenging of free radicals, which ultimately resulted in better survival and growth of *M. rosenbergii* PL. Therefore, these herbs can be utilized for development of sustainable culture of *Macrobrachium*.

**Acknowledgement**

The University Grants Commission (UGC), Government of India, New Delhi is gratefully acknowledged for the financial support provided.

**References**


Drekalusaram S, Herunsalee A, Yoshimizu M, Ezura Y (1996). Protective efficacy of *Clinacanthus nutans* yellow-


Nelson C, Eger KTC (2010). Farm and forestry production and marketing profile for black pepper (*Piper nigrum*).


Pullaiah T (2002). Medicinal plants in Andhra Pradesh (India), Regency publications, New Delhi.


Roe JK, Keuther CA (1943). The determinations of ascorbic acid in whole blood and urine through the 2, 4-dinitrophenylhydrazine (DNPH) derivative of dehydroascorbic acid. *Journal of Biological Chemistry* **147**, 399-407.


