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

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**ABSTRACT**

In this study, *Piper longum* (long pepper), *Piper nigram* (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. nigram* 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 herbals 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.



## 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, herbals 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 *Penaeus 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 scavenge 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, antipyretic, 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, piperine, 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

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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, antihepatotoxic, 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 hypnotic (Nelson & Eger, 2010). Externally it is valued for its rubifacient 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 Edwardisellosis 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 (Mishra, 2010). It is also used as a purgative, an antidote to snake-bite 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, anticancer and immunomodulatory activities (Yusof *et al*. 2002; Sasidharan *et al.* 2010; Famurewa *et al*. 2011). It also used as an antiemetic 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 & Secombes, 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, *M. 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$^o$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. nigram* 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)

$$\text{Feed Conversion Ratio (FCR)} = \frac{\text{Total quantity of feed consumed (dry wt.)}}{\text{Live weight gain (g)}}$$

$$\text{Survival Rate (SR)} = \frac{\text{No. of live prawns}}{\text{No. of prawns introduced}} \times 100$$

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$^0$C for 20 minutes. The supernatant was used as crud 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 $Na_2HPO_4$ and 2 mM $KH_2PO_4$), 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 (Laemlli, 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), carbonic 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.

$$Na^+/K^+ \text{ Content (mg)} = \frac{\text{Sample reading}}{\text{Standard reading}} \times \frac{\text{Standard concentration}}{\text{Sample concentration}} \times \text{Purity of NaCl/ K}$$

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, *P. longum* incorporated feed fed PL showed the best performance followed by *P. nigrum* and *Z. officinale* (Table 1). The increase in growth performance has also previously been reported in *M. rosenbergii* fed with *Ocimium sanctum,* and *Withania somnifera* incorporated feeds (Bhavan *et al*. 2011), *Andrographis paniculata, Cissus quadrangularis,* and *Eclipta alba* incorporated feeds (Shanthi *et al*. 2012) and greens, *Murraya koenigii*, *Coriandrum sativum* and *Menthe arvensis* incorporated feeds (Bhavan *et al*. 2012).

### Digestive enzymes

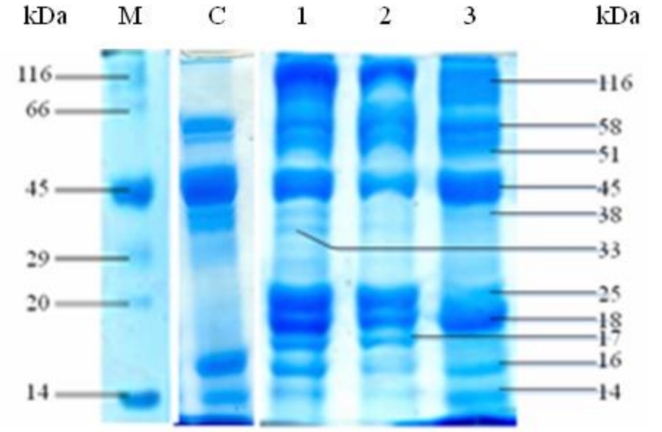
The activities of digestive enzymes, such as protease, amylase and lipase were found to be significantly increased (P<0.05) in experimental feeds fed PL groups when compared with control. Among the three herbs used, *P. longum* incorporated feed fed PL showed the best performance followed by *P. nigrum* and *Z. officinale* (Table 1). Increase in activities of protease, amylase and lipase has also previously been reported in *M. rosenbergii* PL fed with *A. paniculata, C. quadrangularis, E. alba*, *M. koenigii*, *C. sativum* and *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, *P. longum* incorporated feed fed PL showed the best performance followed by *P. nigrum* and *Z. officinale* (Table 1). Increase in total protein has also previously been reported in *M. rosenbergii* PL fed with *O. sanctum, W. somnifera, A. paniculata*, *C. quadrangularis, E. alba, M. koenigii*, *C. sativum* and *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 *P. longum*, *P. nigrum* and *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 14kDa) 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, carbonic anhydrase, soyabean trypsin inhibitor and lysozyme respectively. In *P. longum* incorporated feed fed PL all these bands were stained more intensely than *P. nigram*, and *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 herbals incorporated feeds fed PL. Therefore, it is suggested that these medicinal herbs have their own influence in protein synthesis.




M, marker proteins; C, control; 1, *P. longum* ; 2, *P. nigrum*; 3, *Z. officinale*

**Figure 1: Protein profile of the muscle tissue of *M. rosenbergii* fed with *P. longum*, *P. nigrum* and *Z. officinale* incorporated feeds**

The herbal principles may enhance protein synthesis as reported in *L. rohita* (Johnson & Banerji, 2007) fed with *S. portulacastrum* supplemented diet. The herbal growth promoters may induce transcription and enhanced protein synthesis (Citarasu, 2009; Poonkodi *et al*. 2012).

### 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, *P. longum* incorporated feed fed PL showed the best performance followed by *P. nigrum* and *Z. officinale* (Table 1). Elevation in concentrations of vitamin-C and vitamin-E has previously been reported in *M. rosenbergii* PL fed with *A. paniculata, C. quadrangularis, E. alba*, *M. koenigii*, *C. sativum* and *M. arvensis* incorporated feeds (Shanthi *et al*. 2012; Bhavan *et al*. 2012).

**Table 1: Morphometric data, nutritional indices, biochemical constituents, activities of digestive enzymes, concentration of vitamins and minerals in *M. rosenbergii* PL fed with medicinal herbs (*Z. officinale, P. nigrum* and *P. longum*) incorporated feeds**

| Parameters | | Control | Experiment | | |
|---|---|---|---|---|---|
| | | | *P. longum* | *P. nigrum* | *Z. officinale* |
| Morphometric data | Length (cm) | 2.20±0.10 | 4.36±0.28 | 3.84±0.01 | 3.27 ±0.06 |
| | Weight (g) | 0.20±0.01 | 0.66±0.02 | 0.52±0.01 | 0.39±0.01 |
| | Weight gain (g) | 0.131±0.09 | 0.592±0.09 | 0.446±0.07 | 0.316±0.08 |
| Nutritional indices | Survival rate (%) | 73.33±0.65 | 96.66±0.14 | 90.00±0.8 | 83.33±1.00 |
| | Specific growth rate | 0.211±0.10 | 0.723±0.09 | 0.616±0.08 | 0.491±0.05 |
| | Feed conversion ratio | 4.8±0.96 | 1.92±0.23 | 2.4±0.67 | 3.03±0.89 |
| Digestive enzymes (U/ mg protein) | Protease | 1.33±0.32 | 4.83±0.25 | 4.38±0.04 | 4.37±0.01 |
| | Amylase | 1.72±0.27 | 5.32±0.65 | 3.50±0.50 | 3.46±0.65 |
| | Lipase | 0.15±0.07 | 0.70±0.10 | 0.68±0.01 | 0.67±0.17 |
| Total protein (mg/g wet. wt) | | 91.02±193 | 423.66±0.04 | 337.6±4.00 | 344.97±1.97 |
| Vitamins (µmol/mg protein) | Vitamin C | 24.00±1.00 | 58.70±2.28 | 50.01±2.10 | 48.49±0.50 |
| | Vitamin E | 26.50±0.50 | 46.38±1.49 | 39.83±0.17 | 37.04±1.04 |
| Minerals (mg/g) | $Na^+$ | 5.44±0.98 | 19.52±1.38 | 17.17±0.98 | 16.26±0.88 |
| | $K^+$ | 4.07±1.01 | 11.74±0.80 | 8.62±1.90 | 7.67±056 |

Initial length: 1.56±0.08 cm; Initial weight: 0.074±0.02 g.

Length and weight were randomly measured from five individuals and the mean value was considered as a single observation and three such observations were made.

Each value is mean ± SD of three individual observations.

All the values are significant at P<0.05

Increase in concentrations of $Na^+$ and $K^+$ has also previously been reported in *M. rosenbergii* PL fed with *Alteranthera 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 scavenge reactive radicals, such as hydroxyl, perhydroxyl, peroxyl 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 & Trabor, 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*.

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