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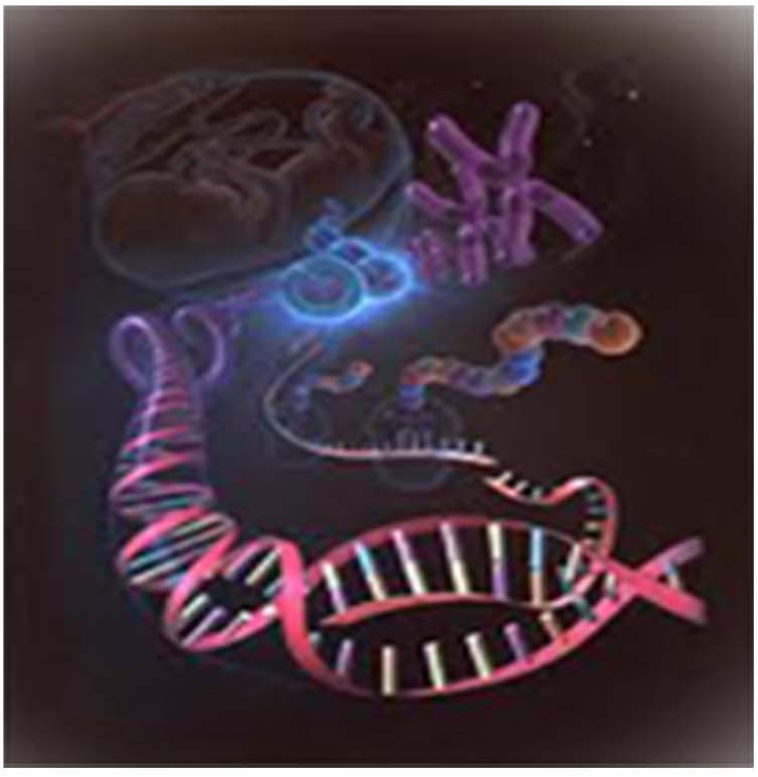

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Research Paper

EFFECTS OF *MYRISTICA FRAGRANS*, *GLYCYRRHIZA GLABRA* AND *QUERCUS INFECTORIA* ON GROWTH PROMOTION IN THE PRAWN *MACROBRACHIUM ROSENBERGII*

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Objective: To understand whether the medicinal herbs, *Myristica fragrans* (nutmeg) *Glycyrrhiza glabra* (liquorice) and *Quercus infectoria* (gallnut) have effects on growth promotion in the post larvae (PL) of the prawn, *Macrobrachium rosenbergii*. Methods: *M. fragrans* (seed powder), *G. glabra* (stem powder) and *Q. infectoria* (fruit powder) were incorporated with basal diet at three different concentrations (1%, 3%, and 5%) individually and fed to the PL of *M. rosenbergii* (1.56±0.18 cm; 0.074± 0.02 g) for a period of 60 days under triplicate experimental set-up. Results: Significant elevations ($P < 0.05$) in weight gain, survival rate, activities of digestive enzymes (protease, amylase and lipase), concentrations of total protein, non-enzymatic antioxidants (vitamins C and E) and minerals (Na^+ and K^+) were recorded in *M. fragrans* incorporated feed fed PL followed by *G. glabra* and *Q. infectoria* when compared with control. Nine polypeptide bands of molecular weight between 116-14 kDa were resolved in the muscle tissue of PL. These bands were stained more intensely in experimental PL when compared with control. Conclusion: These herbs have the ability to induce secretion of protease, amylase and lipase in *M. rosenbergii* PL, which lead to increased food consumption and absorption of nutrients, and resulted in elevation of total protein, vitamins and minerals.

Keywords: Prawn, Nutmeg, Liquorice, Gallnut, Growth, Digestive enzymes, Vitamins, Protein

INTRODUCTION

Aquaculture is the production of aquatic plants and animals under the controlled or semi controlled conditions (Stickney *et al.,* 2000). It is one of the fastest growing animal food sectors and provides over 13% of the animal protein for the human consumption (WHO, 2003; FAO, 2010). In addition to contributing to global food production, aquaculture is a major economic activity and an important source of foreign exchange for several developing countries. Currently aquaculture supplies about 50% of the global demand for fish and fishery products with about 90% of the products coming from the Asian region (FAO, 2009). The production of carp and

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shrimps (average national production, 8-12 tons/ha/year) addresses various issues like food and nutritional security, employment, livelihood support and socioeconomic status of fishing communities (Tripathi, 2003; Pillai and Katiha 2004; FAO, 2010). Among the world seafood, prawns and shrimps contribute about 20% by volume. *Macrobrachium rosenbergii* is considered to be one of the aquaculture species with increasing potential and thus was introduced worldwide (Sharshar and Azab, 2008; Radheyshyam, 2009). China, Thailand, India and Bangladesh are the top producers of this species with an annual production of over 30,000 tons have been achieved through monoculture practices (FAO, 2010). *M. rosenbergii* is nutritious with good source of protein, amino acids, fatty acids and carbohydrate, and considerable quantity of lipid, minerals, vitamins and fiber as well (Bhavan *et al.,* 2010a).

In aquaculture of crustaceans, the artificial feed sector has made tremendous development in production of sustainable and nutritious feeds (Chandrapal, 2005). The quality of supplementary feeds forms determining criteria in the yield and profit. The cost of feed is the single largest and most important operating expense. It is feasible to use cheap and easily available ingredients, which are palatable and have high growth efficiency. In addition to cheaply available animal (fish meal, poultry meal and blood meal, etc.) and plant byproducts (cereals, pulses, soybean meal, groundnut oilcake, rice brawn, sunflower oil, coconut oil and linseed oil, etc.), vegetable waste, fruits waste, greens, and herbals are also used as supplementary feed ingredients for better survival and growth (Bhavan *et al.,* 2010b; Rebecca and Bhavan, 2011; Bhavan *et al.,* 2011a, b; Bhavan and Radhakrishnan, 2012; Shanthi *et al.,* 2012; Kavithaa *et al.,* 2012; Poongodi *et al.,* 2012; Bhavan *et al.,* 2012; and Muralisankar and Bhavan, 2013). The medicinal plants have phytochemicals, the bioactive substances which are responsible for the biological activities they exhibit, for example the protection of health against chronic degenerative diseases (Fukumoto and Mazza, 2000). The herbal biomedicine active principles in the aquaculture have the characteristics of growth promoting ability, tonic to improve the immune system, anti-microbial capability, anti-stress characteristics and stimulating appetite due to presence of alkaloids, flavonoids, pigments, phenolics, terpenoids, starch, steroids and essential oils. Furthermore, the uses of herbal medicines will reduce the applications of synthetic antibiotics, hormones etc. (Citarasu *et al.,* 2002; Sivaram *et al*., 2004; Citarasu, 2010). In the present study, three medicinal herbs, such as *Myristica fragrans* (Family: *Myristicaceae*; English name: Nutmeg; Tamil name: *Jathikkai*; Part used: Seed powder) *Glycyrrhiza glabra* (Family: *Papilionaceae*; English name: Liquorice; Tamil name: *Athimaduram*; Part used: Stem powder) and *Quercus infectoria* (Family: *Fagaceae*; English name: Gallnut; Tamil name: *Massikkai*; Part used: Fruit powder) were incorporated with feed as supplements. This was to understand their effects on growth promotion, induction of digestive enzymes activities (protease, amylase and lipase) and contents of total protein, vitamins (C and E), minerals (sodium, Na^+ and potassium, K^+) and profiles of protein in *M. rosenbergii* post larvae, as they have some phytochemicals. The active principle of *M. fragrans* is reported as myristicin, myristic acid and essential oil (Akinboro *et al.,* 2011). *G. glabra* contains formononetin, glabrone, neoliquiritin and

hispaglabradin (Akram *et al.,* 2011). *Q. infectoria* has tannins, phenol and carboxylic acid (Umachigi *et al.,* 2008).

Chirathaworn *et al.* (2007) reported that *M. fragrans* act as an anticancer agent as it contains myristicin, which has cytotoxic and apoptotic effects in human neuroblastroma SK-N-SH cells with an accumulation of cytochrome and activation of caspase-3 in the cytosol. The methanolic extracts of *M. fragrans* promoted the immunity and reduced the load of *Vibrio harveyi* in the estuarine giant grouper, *Epinephelus tauvina* (Sivaram *et al.,* 2004). It has antibacterial, antidiarrhoeal and abortifacient potential (Akinboro *et al.,* 2011).

Historically, the dried rhizome and root of *G. glabra* were employed medicinally by the Egyptian, Chinese, Greek, Indian, and Roman civilizations as an expectorant and carminative. In modern medicine, licorice extracts are often used as a flavoring agent to mask bitter taste in preparations, and as an expectorant in cough and cold preparations (Akram *et al.,* 2011). Licorice extracts have been used for more than 60 years in Japan to treat chronic hepatitis, and also have therapeutic benefit against other viruses, including Human Immunodeficiency Virus (HIV), Cytomegalovirus (CMV), and Herpes simplex. Deglycyrhizinated licorice (DGL) preparations are useful in treating various types of ulcers, while topical licorice preparations have been used to sooth and heal skin eruptions, such as psoriasis and herpetic lesions. In aquaculture, the common carp *Cyprinus carpio* and large yellow croaker *Pseudosciaena crocea* showed improvement in immunity and total protein content when fed with a feed contained *Astragalus membranaceus*, *Polygonatum multiflorum*, *Isatis tinctoria* and *G. glabra*. (Yuan *et al.*, 2007). Kumar *et al.* (2007) reported that the roots of *G. glabra* mixed with basal diet produced higher growth rate in *Cirrhinus mirigala.*

Q. infectoria significantly increased the serum HDL-cholesterol level with a concomitant increase in both serum glucose and insulin levels in rat, and the extract showed anti-bacterial, anti-inflammatory and anti-ulcerogenic activities (Umachigi *et al.,* 2008). The herbal extracts of *Murraya koeniji*, *Psoralea corylifolia and* Q. *infectoria* reported to suppress the pathogenic microflora, such as *Salmonella* sp., *Proteus* sp., *Yersinia* sp., and *Aeromonas* sp., in the gut of Indian white shrimp, *Fenneropenaeus indicus* (Citrasu *et al.,* 2010). According to Velmurugan *et al.* (2010), *Adatoda vasika*, *Murraya koenigii, Ocimum basilicum, Psoralea corylifolia* and Q. *infectoria* has effectiveness against pathogens, and survival of *Penaeus monodon* post larvae.

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, Tamil Nadu, 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 (25 g), groundnut oil cake (25 g), and wheat bran (10 g). Tapioca flour (7 g) and egg albumin (5 mL/ 100 g) was added as binding agents. Before addition of the egg albumin the mix was cooked for 15 min 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, dried seed of *M. fragrans*, dried stem of *G. glabra* and dried fruit of *Q. infectoria* was separately added in three different extra concentrations (1 g, 3 g and 5 g extra/ 100 g) 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* (1 g 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 min. 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 1 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), soyabean 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 and 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.

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

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

In the present study, Indian native herbs, *M. fragrans, G. glabra* and *Q. infectoria* incorporated feeds fed *M. rosenbergii* PL showed better result when compared with control.

Morphometry and Survival Data

The length, weight, weight gain, food conversion ratio (FCR) and survival data are given in Table 1. Values of these parameters except FCR were found to be significantly increased ($P < 0.1$) in experimental feeds fed PL groups when compared with control. The reverse trend was seen in FCR, which indicates the quality of the feed prepared. Among the three herbs used, *M. fragrans* incorporated feed fed PL showed the best performance followed by *G. glabra* and *Q. infectoria*. This was in the order of 5% incorporation > 3% > 1% in all the herbs tested. Therefore, among the three concentrations tested in each green, 5% incorporation was found to be produced the best result. Actually, there was progressive increase between 1% and 3%, and between 3% and 5% incorporations of each herb (Table 1).

The increase in growth performance has also previously been reported in *M. rosenbergii* fed with 'Nutripro-aqua', the herbal based diet (Keshavanth and Jayaram, 1998), *Ocimium sanctum,* and *Withania somnifera* incorporated feeds (Bhavan *et al.,* 2011b), *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. In *Penaeus indicus* fed with 'Livol IHF-1000' a diet containing *Boerhavia diffusa, Solanum nigrum* and *Terminalia arjuna* (Sambhu and Jayaprakash, 2001). In *Penaus monodon* fed with *W. somnifera* and *Mucuna pruriens* extracts enriched *Artemia*

Table 1: Growth, Survival, Total Protein Content, Activities of Digestive Enzymes, Concentrations of Vitamins and Minerals in Different Herbal Incorporated Feeds Fed *M. rosenbergii* PL

Parameters	Control	*M. fragrans*			*G. glabra*			*Q. infectoria*		
		1%	3%	5%	1%	3%	5%	1%	3%	5%
Feed Leaching (%), 2 h	10±1.5	12±1.5*	14±1.6	14±1.5*	15±1.6	15±1.5*	16±1.5*	14±1.4	16±1.5*	16±1.5*
Length (cm) (Initial, 1.56±0.18)	2.20±0.16	3.02±0.30	3.08 ±0.40	4.30 ±0.45	2.76 ±0.40	2.80 ±0.30	3.62 ±0.40	2.50 ±0.23	3.10 ±0.34	3.34 ±0.38
Weight (g) (Initial, 0.074±0.02)	0.20±0.04	0.27±0.07^{NS}	0.38±0.09	0.44 ±0.08	0.26 ±0.06^{NS}	0.34 ±0.07	0.40 ±0.06	0.26 ±0.05^{NS}	0.30 ±0.05	0.38 ±0.05
Weight Gain	0.13±0.02	0.20±0.07^{NS}	0.31±0.09	0.37 ±0.08	0.19 ±0.06^{NS}	0.27 ±0.07	0.33 ±0.06	0.19 ±0.05^{NS}	0.26 ±0.07	0.31 ±0.05
Food Conversion Ratio	4.68±0.72	3.55±1.32^{NS}	2.22±0.67	1.95 ±0.43	3.58 ±0.98	2.52 ±0.67	2.16 ±0.40	3.67 ±1.21^{NS}	2.91 ±0.65	2.29 ±0.37
Survival Rate	80.0±3.0	86.0±2.0	93.0±4.0	96.0 ±4.0	83.0 ±4.0	93.0 ±4.0	96.0 ±4.0	83.0 ±2.0	89.0 ±5.0	90.0 ±5.0
Total Protein (mg/g wet wt.)	91.02 ±4.72	174.87 ±6.50	223.66 ±6.86	242.97 ±6.97	149.32 ±5.55	194.87 ±6.05	224.43 ±6.96	147.84 ±4.64	159.32 ±4.51	174.87 ±5.50
Protease (U/mg protein)	4.50±0.74	7.36±1.15	7.55 ±1.14	8.95 ±1.16	6.29 ±0.90	7.39 ±1.19	7.82 ±1.16	5.47 ±1.13	6.32 ±1.09	7.44 ±1.15
Amylase (U/mg protein)	1.06±0.11	1.64±0.19	2.11 ±0.14	2.54 ±0.15	1.22 ±0.15	1.81 ±0.19	2.25 ±0.14	1.19 ±0.12	1.38 ±0.13	2.09 ±0.17
Lipase (Unit x 10^3) (U/mg protein)	0.47±0.07	0.61±0.06	0.85 ±0.05	1.10 ±0.08	0.50 ±0.05^{NS}	0.83 ±0.04	0.95 ±0.05	0.44 ±0.06	0.71 ±0.07*	0.80 ±0.09
Vitamin C (μmol./mg protein)	25.08±1.33	39.50±1.23	43.21 ±1.38	66.98 ±2.35	36.82 ±1.11	40.10 ±1.31	51.91 ±2.29	34.80 ±1.43	38.88 ±1.14	49.86 ±1.21
Vitamin E (μmol./mg protein)	26.53±1.80	39.47±1.77	50.53 ±2.06	57.58 ±2.16	34.27 ±1.25	46.84 ±1.26	53.65 ±2.16	32.00 ±1.49	45.28 ±1.88	50.19 ±2.04
Na^+ (mg/g)	0.13±0.02	0.18±0.04	0.34 ±0.07	0.54 ±0.09	0.17 ±0.06^{NS}	0.30 ±0.05	0.42 ±0.08	0.14 ±0.02*	0.22 ±0.04	0.35 ±0.08
K^+ (mg/g)	0.25±0.05	0.37±0.06	0.42 ±0.09	0.59 ±0.10	0.32 ±0.07	0.36 ±0.09	0.55 ±0.13	0.24 ±0.06^{NS}	0.32 ±0.09^{NS}	0.50 ±0.10

Note: 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; ^{NS}, Not statistically significant; *'*t*' and significance can't be calculated because of the deviations in control and treatment are the same; All other values are significant at $P<0.05$.

(Babu *et al.,* 2008), individual herbs such as, *Hygrophila spinosa, W. somnifera, Zingiber officinalis, Solanam trilobatum, A. paniculata* and *Psoralea corylifolia* enriched *Artemia* (Citarasu *et al.,* 2002) and the herbal product, 'Tefroli' made of *Tephrosia purpurea, E. alba*, *Phyllanthus niruri, A. paniculata, O. sanctum* and *Terminalia chebulam* enriched *Artemia* (Citarasu, 2010).

Digestive Enzymes

The activities of digestive enzymes, such as protease, amylase and lipase are also given in Table 1. Activities of these enzymes were found to be significantly increased ($P < 0.05$) in experimental feeds fed PL groups when compared with control. Among three herbs tested, *M. fragrans* incorporated feed fed PL showed the maximum increased activity at 5% incorporation followed by 3% and 1%, followed by *G. glabra* and *Q. infectoria* in the same trends as recorded for *M. fragrans*. Therefore, 5% incorporation of each herb was found to be the best in enhancing the activities of these digestive enzymes.

Increase in activities of protease, amylase and lipase has also previously been reported in *M. rosenbergii* PL fed with *A. paniculata, C. quadrangularis,* and *E. alba* incorporated feeds (Shanthi *et al.,* 2012) and *M. koenigii*, *C. sativum* and *M. arvensis* incorporated feeds (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. Papaya leaf meal contains an enzyme, papain, which was reported to increase protein digestion, food conversion ratio, specific growth rate and weight gain in *P. monodon* PL (Penaflorida, 1995). 'Livol (IHF-1000)' a herbal growth promoter containing *B. diffusa, S. nigrum, T. arjuna,* Colosynth and black salt has been reported to increase food consumption and digestion, thereby leading to better health and production in fishes (Bolle, 1996; Jayaprakas and Euphrasia, 1996) and *P. indicus* PL (Sambhu and Jayaprakesh, 2001). *Z. ofcinalis* enriched *Artemia* was reported to improve activities of digestive enzymes, amylase, protease and lipase in *P. monodon* PL (Venketramalingam *et al.,* 2007). It has also been reported that amylase and cellulase activities in *M. rosenbergii* were much higher than those found in the marine species, *P. monodon*, *Penaeus japonicus*, and *Penaeus penicillatus* (Chuang *et al.,* 1985).

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 (Table 1). Among three herbs tested, *M. fragrans* incorporated feed fed PL showed the best result (5% incorporation >3% >1%), followed by *G. glabra* and Q. *infectoria* in the same trends as recorded for *M. fragrans*.

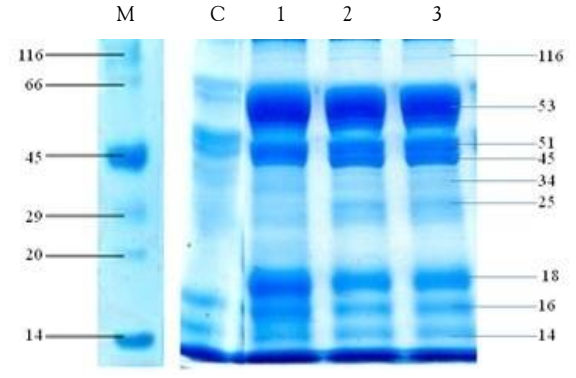
Increase in total protein has also previously been reported in *M. rosenbergii* PL fed with *O. sanctum,* and *W. somnifera* incorporated feeds (Bhavan *et al.,* 2011b), *A. paniculata, C. quadrangularis,* and *E. alba* incorporated feeds (Shanthi *et al.,* 2012) and *M. koenigii*, *C. sativum* and *M. arvensis* incorporated feeds. The herbal principles may enhance protein synthesis as reported in *L. rohita* (Johnson and Banerji, 2007) fed with *S. portulacastrum* supplemented diet. The herbal growth promoters helped to induce transcription, leading to increased RNA, which in turn coupled with increased amino acid and finally

enhanced protein synthesis (Citarasu, 2010; Poonkodi *et al.,* 2012).

Protein Profile

Polypeptide bands of molecular weight between 116-14 kDa were resolved in the muscle tissue of PL fed with *M. fragrans*, *G.glabra* and *Q. infectoria* incorporated feeds, and control as well (Figure 1). There were nine Coomassie blue stained protein bands (116, 53, 51, 45, 34, 25, 18, 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 α-galactosidase, Bovine serum albumin, ovalbumin, carbonic anhydrase, soyabean trypsin inhibitor and lysozyme, respectively. Generally, bands resolved in all the regions in test groups were stained more intensely when compared with control. Among the test groups the increased staining intensity was found to be very obviously higher in *M. fragrans* incorporated feed fed PL followed by *G. glabra* and *Q. infectoria*. In the test categories the staining intensity of various polypeptide bands was in the order of 53>51>45>18>16>14>others.

Figure 1: Protein Profile of *M. rosenbergii* (Muscle Tissue) Fed with *M. fragrans, G. glabra* and *Q. infectoria* Incorporated Feeds



Note: M – marker proteins; C – control; 1 – M. *fragrans*; 2 – G. *glabra*; 3 – *Q. infectoria*.

This trend was not matched with control (51>45>16>14>53>others. Therefore, it is suggested that these medicinal herbs have their own influence in protein synthesis.

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 three herbs tested, *M. fragrans* incorporated feed fed PL showed the best result in vitamins (C and E) and minerals (Na^+ and K^+) levels (5% incorporation >3% >1%), followed by *G. glabra* and *Q. infectoria* in the same trends as recorded for *M. fragrans*.

Elevation in concentrations of vitamin-C and vitamin-E has previously been reported in *M. rosenbergii* PL fed with *A. paniculata, C. quadrangularis,* and *E. alba* incorporated feeds (Shanthi *et al.,* 2012), and *M. koenigii*, *C. sativum* and *M. arvensis* incorporated feeds (Bhavan *et al.,* 2012). Ascorbic acid is a potent antioxidant, which scavenges reactive radicals such as hydroxyl, perhydroxyl, peroxyl and nitric oxide (Halliwell and Gutteridge, 2001; Bendich *et al.,* 1986; and Karakoe *et al.,* 1997). Ascorbic acid is believed to regenerate vitamin E from its oxidized form (Wells *et al.,* 1990) thereby raises the antioxidant status. In the present study, the increased contents of these vitamins clearly indicated the fact that the general health of PL was improved due to incorporation of *G. glabra*, *Q. infectoria* and *M. fragrans*.

Dietary supplementation of Chinese herbal extracts reported to enhance the antioxidant ability in fishes (Rao *et al.,* 2006; Christybapita *et al.,* 2007; and Xie *et al.,* 2008). In crustaceans, a few

studies suggested similar effects (Alava *et al.,* 1993a, b; Cahu *et al.,* 1995). Some crustaceans have a limited ability to synthesis ascorbic acid but this is considered insufficient to meet metabolic requirements (Conklin, 1997). Therefore, its supplementation is important. Vitamin E (α-tocopherol) acts as scavenger of free radicals and supports the antioxidant enzyme system (Tocher *et al.,* 2002; Van der meeren *et al.,* 2008). It reduces peroxyl radicals in membrane lipids and prevents lipid peroxidation, thus protects the cell and organelle membranes (Burton and Trabor, 1990), and is therefore crucial for normal development of tissues, including cartilage and bone (Lal and Lewis-McCrea, 2007). In this study, the elevation recorded in vitamin C and E in *M. rosenbergii* PL fed with herbal incorporated diets ensures protection and favors better survival and growth.

The macro element sodium in animal is connected with the regulation of osmotic pressure and the maintenance of acid-base balance. It has an effect on muscle irritability, and plays a specific role in the absorption of carbohydrate. Potassium is an important element for growth of animal. It has major action in intracellular fluid, and regulates intracellular osmotic pressure and acid-base balance. It is required for glycogen and protein synthesis, and the metabolic breakdown of glucose. In a study, Shiau and Jia-Fen (2001) suggested that weight gain of shrimp was improved due to the increased level of potassium in the diet. Like sodium, potassium has a stimulating effect on muscle irritability. 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).

CONCLUSION

M. fragrans, G. glabra and *Q. infectoria* have the ability to induce secretion of protease, amylase and lipase in *M. rosenbergii* PL, which lead to increased food consumption and absorption of nutrients, which associated with active principles, and resulted in elevation of total protein, vitamins and minerals. The elevated level of vitamin-C and E promotes general health and aided for better growth and survival of experimental PL. This study offers promising possibilities of using these herbs in sustainable development of freshwater prawn culture.

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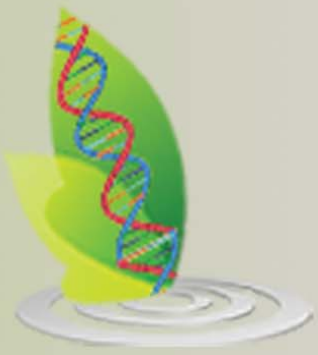

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