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GROWTH PROMOTION AND SURVIVAL ENHANCEMENT OF THE FRESHWATER PRAWN *MACROBRACHIUM ROSENBERGII* POST LARVAE FED WITH *ALLIUM SATIVUM, ZINGIBER OFFICINALE* AND *CURCUMA LONGA*

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ABSTRACT

Post Larvae (P.L.) of *M. rosenbergii* were fed with ten individually formulated diets using *A. sativum* (A.S.), *Z. officinale* (Z.O.) and *C. longa* (C.L.) at three concentrations (1%, 3% and 5%) separately, for a trial of 90 days. Each diet contained an herb supplemented at any one of the given concentration. Diet without any of the above mentioned herbs served as control. At the end of the trial, analyses were done on growth, survival rate, feed quality, nutritional, biochemical and energy utilization indices. 5% Z.O. offered $P < 0.046$ level of increase in weight gain (WG) and daily growth rate (DGR), $P < 0.080$ level of increase in total proteins, amino acids, carbohydrates, lipids and vitamin-E, $P < 0.638$ level of increase in feed quality indices such as digestibility (D) %, gross feed conversion ratio (GFCR) %, net feed conversion ratio (NFCR) %, feed efficiency (FE) and $P < 0.854$ level of increase in nutritional indices such as protein intake (PI), amino acid intake (AI), carbohydrate intake (CI), lipid intake (LI), protein efficiency ratio (PER), protein conversion ratio (PCR), protein assimilation rate (PAR) and protein productive value (PPV) was also obtained with 5% Z.O. 5% C.L. increased the survival rate (SR) at $P < 0.049$ and also the tissue levels of vitamin-C, iron, sodium and potassium at $P < 0.129$. 5% A.S. increased the tissue level of phosphorus at $P < 0.082$. The feed conversion ratio (FCR) was found to be higher at $P < 0.341$ level in the group fed with control diet. $P < 0.084$ level of increase in rate of feeding, absorption, conversion and ammonia excretion of the prawns was obtained with 5% Z.O. The diets were analyzed for minerals and non-enzymatic antioxidants. $P < 0.240$ level of increase in vitamin-C, flavanoids, iron, sodium and potassium were recorded in 5% C.L. and $P < 0.089$ level of increase in vitamin-E was recorded in 5% Z.O., also $P < 0.530$ level of increase in phosphorus was recorded in 5% A.S. Increased feed intake and a better quality of the feed has resulted in better nutritional status of the diet-fed animals. Z.O. improved feed intake and promoted the growth. C.L. served as a good source of non-enzymatic antioxidants and enhanced the survival of the freshwater prawns. Therefore, it is suggested that these herbs can be used as cheap and safer alternatives against synthetic hormones and antibiotics for its growth and survival.

Keywords: Aquaculture, Aquafeed, Antioxidants, Nutrition, Energy.

INTRODUCTION

Aquaculture provides a good opportunity for developing countries to gain a foothold in the world food market (Manoj and Vasudevan, 2009; Ahmed *et al*., 2013; Hossain *et al*., 2013). As the largest species of its genus, *M. rosenbergii* has been widely used in aquaculture and has thus been introduced throughout most of the tropical and temperate regions of the world (Mather and de Bruyn, 2003). There are several reports available with respect to improvement of nutritional quality of *M. rosenbergii* under culture conditions (Hasanuzzaman *et al*., 2009; Bhavan *et al*., 2010).

Feed formula is a major input in the hatchery, the availability of cost-effective feeds play an important role in aquaculture industry (Chunchom *et al*., 2010). The production and studies on the biochemical composition of edible organisms are important from the nutritional point of view (Soundarpandian and Ananthan, 2008). The evaluation of feed ingredients is crucial to nutritional research and feed development for aquaculture species (Glencross

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et al., 2007). The use of artificial feeds with optimized nutritional quality is the need in aquaculture. Success in aquaculture depends to a great extent on sound nutritional practices based on the knowledge of nutrients required by the species cultured. Supplementation of diets with growth inducing substances has the potential to be profitable because of the improved growth rate or reduced culture period (Keshavanath *et al*., 2003).

In aquaculture, infectious diseases are a major problem, causing heavy loss to fish farmers (Logambal *et al*., 2000). The use of hormones, antibiotics, vitamins and several other chemicals is rather difficult, non-effective and costly and also involves environmental hazards. Even though they give positive effects, they have residual and other side effects. Because of their safety to fish farmers, the consumer, and the environment as well as their recognized antimicrobial activity, plant essential oils have been suggested as possible alternatives to antibiotics to control disease in aquatic animals (Rattanachaikunsopon and Phumkhachorn, 2009). Many plants and plant products have already been investigated for their therapeutic and prophylactic effects on several fish diseases (Abutbul *et al*., 2004; Pachanawan *et al*., 2008).

A better understanding of growth in aquaculture presents significant benefits in terms of productivity, sustainability and profitability. Natural antioxidants are a wide class of compounds coming mainly from spices, herbs and recently by agriculture by-product (Moure *et al*., 2001). Several of these antioxidants are utilized for improving animal health (Sutton *et al*., 2006) for organic animal production and for improving the quality of final product (Hamre *et al*., 2004).

Energy is not a nutrient, but released during metabolic oxidation of carbohydrates, lipids and amino acids (Sales, 2009). The nutritional value of a feed ingredient is based not solely on its chemical composition, but also on the amount of nutrients and energy that the animal can absorb and use from it. In crustaceans, ingested food energy is primarily channel into growth, metabolic maintenance, ammonia excretion, feces and moulting (Bhavan *et al*., 2010). Therefore, evaluation of energy utilization by experimental prawns following influence of medicinal herbs is also needed.

Herbs are currently used in commercial aquaculture as growth-promoting substances, antimicrobial agents, nutrients as well as many other applications. Garlic (*Allium sativum*) has acquired a reputation in Asiatic and Western cultures as a prophylactic and therapeutic medical agent (Song and Milner, 2001). Turmeric (*Curcuma longa*) has been used as a cheap, commercial bioresource as fish additive in fish feed (Mukherjee *et al*., 2009). Ginger (*Zingiber officinale*) has been recommended for use as carminative, diaphoretic, antispasmodic, expectorant, peripheral circulatory stimulant, astringent, appetite stimulant, anti-inflammatory agent, diuretic and digestive aid (Kim *et al*., 2007). Efficient feed intake and nature of the constituting feed ingredients could also reduce ammonia toxicity in the culture system, as ammonia is the main nitrogenous product excreted by crustaceans and additionally is produced from the ammonification of organic matter in a culture system (Frias-Espericueta *et al.,* 2000). Hence there is a need to depend on medicinal plants for growth promotion, survival and ammonia stress tolerance.

The aim of the present investigation is to first test the suitability of the selected diets for use as feed for *M. rosenbergii* post larvae so as to test for any adverse effects in the experimental groups against the control group and then to analyze for the favorable qualities like growth promotion, survival enhancement, etc. Thereby these natural and readily available medicinal plants can be used for digestive and immune enhancem1ents and anti-stress properties which could limit ammonia toxicity, etc. as an alternative for highly priced, environmental contaminating and consumer's hazardous synthetic compounds like antibiotics, growth hormones, appetitizers, etc.

MATERIALS AND METHODS

Formulation, preparation and analysis of Diets

Ten different diets were prepared (Table 1). Diet-1 without any herb supplementation served as control diet. Diet-2, Diet-3 and Diet-4 contained 1%, 3% and 5% of A.S. powder respectively and Diet-5, Diet-6 and Diet-7 contained 1%, 3% and 5% of C.S. powder respectively. Diet-8, Diet-9 and Diet-10 contained 1%, 3% and 5% of Z.O. powder respectively. The remaining proportions

(%) are those of the basal ingredients. Feed ingredients were weighed out and grounded to a fine powder separately for good binding capacity as per the proportion and were mixed thoroughly for uniform distribution of the nutrients as per the formulation. Mixed feed ingredients were subjected to steam cooking to improve the binding capacity as well as the digestion by steaming at 100 ºC for 5 minutes. They were brought to the room temperature, to which herbal powder, egg albumin, vitamin mix and cod liver oil was added proportionately and was made into thick dough which was immediately squeezed out manually into pellets using a hand pelletizer of 3 mm pore-diameter. Fresh de-skinned *A. sativum* (garlic) bulbs, *Z. officinale* (ginger) and *C. longa* (turmeric) rhizomes were dried, powered and added separately at three different proportions (1%, 3% and 5%) to the basal ingredients comprising of 38 % fish meal, 40% groundnut oilcake, 3% coconut oilcake, 3% wheat barn, 3% rice bran and 3% maize bran, 2% tapioca flour, 1% vitamin and 7% egg albumin. Pellets were sun dried for about three days and were stored in air tight containers to attain maximum shelf-life of the diets.

In these feeds, concentration of minerals and non-enzymatic antioxidants such as iron and phosphorus (Raghuramulu *et al.,* 2003), sodium and potassium (Vogel's method, 1989), Vitamin-C (Roe and Kuether, 1943), Vitamin-E (Baker *et al.,* 1980) and flavanoids (Kumar *et al*., 2008) were estimated.

Table-1. Composition of formulated test diets.

Ingredients (g)	Feeds Experimental									
	Control feed-1	Feed -2 (1% A.S.)	Feed -3 (3% A.S.)	Feed -4 (5% A.S.)	Feed -5 (1% C.L.)	Feed -6 (3% C.L.)	Feed -7 (5% C.L.)	Feed -8 (1% Z.O.)	Feed -9 (3% Z.O.)	Feed -10 (5% Z.O.)
Fish meal	38	37.62	36.86	36.1	37.62	36.86	36.1	37.62	36.86	36.1
Groundnut oilcake	40	39.6	38.8	38	39.6	38.8	38	39.6	38.8	38
Coconut oilcake	3	2.97	2.91	2.85	2.97	2.91	2.85	2.97	2.91	2.85
Rice bran	3	2.97	2.91	2.85	2.97	2.91	2.85	2.97	2.91	2.85
Wheat bran	3	2.97	2.91	2.85	2.97	2.91	2.85	2.97	2.91	2.85
Maize bran	3	2.97	2.91	2.85	2.97	2.91	2.85	2.97	2.91	2.85
Tapioca flour	2	1.98	1.94	1.90	1.98	1.94	1.90	1.98	1.94	1.90
Egg albumin	7	6.93	6.79	6.65	6.93	6.79	6.65	6.93	6.79	6.65
Vitamin mix*	1	0.99	0.97	0.95	0.99	0.97	0.95	0.99	0.97	0.95
B.I. total	100	99	97	95	99	97	95	99	97	95
Medicinal plant supplementation	0	1	3	5	1	3	5	1	3	5
Total feed ingredients	100	100	100	100	100	100	100	100	100	100

**B.I.- Basal Ingredients.

Growth trial and biochemical analysis

P.L.- 15 of the freshwater prawn, *M. rosenbergii* with an average initial body weight of 0.1 g were purchased from the Rosen fisheries, Thrissur, Kerala, India. They were transported to the laboratory in polythene bags filled with oxygenated water and maintained in a cement tank. The larvae 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.

Ten groups of 90 prawns each were selected. Each group was housed in an aquarium of 75 L capacity, allowed to acclimatize for a week and maintained as described previously. From each group 10 prawns were randomly taken for measurements of initial morphometric data and three such measurements were taken (totally 30 P.L. were measured). The resulted mean values

were taken to calculate an average mean value. After measurement these prawns were re-introduced into the respective aquaria, for the estimation of initial biochemical constituents, such as total protein, amino acid, carbohydrate and lipid. Tissues from 30 prawns in each group were pooled separately and taken for analyses. Thus, three such observations were made for each parameter. The remaining 60 prawns in each group were equally divided and housed in three aquaria of 20 L capacity in order to conduct the experiment in triplicate. The feeding trial was conducted for a period of 90 days.

At the end of the feeding trial, proximate compositions of the major biochemical constituents (total protein, lipid, carbohydrate, amino acid, moisture and ash) and trace elements (vitamin-C, vitamin-E, sodium, potassium, iron and phosphorus) were calculated. Indices of growth (Valberde *et al*, 2008) such as Survival Rate (SR), Weight Gain (WG), Daily Growth Rate (DGR), Feed Conversion Ratio (FCR), Gross Feed Conversion Ratio (GFCR) and Net Feed Conversion Ratio (NFCR) were studied.

Studies on protein and other major nutritional indices (Easterson, 1987; Palavesam *et al*, 2008) such as Digestibility (D), Feed Efficiency (FE), Feed Intake (FI), Protein Intake (PI), Amino acid Intake (AI), carbohydrate Intake (CI), lipid Intake (LI), Protein Efficiency Ratio (PER), Protein Conversion Ratio (PCR) were also carried out. Parameters were calculated by using the following formulae so as to evaluate the efficacy of feeds prepared.

Survival Rate (SR) % = No. of live animals / No. of animals introduced X 100

Weight Gained (WG) = Final weight - Initial weight

Daily growth rate (DGR) = Weight Gained / No. of days X 100

Digestibility (D) % = Amount of food consumed - Amount of feces / Amount of food consumed X 100

Food Conversion Ratio (FCR) = Feed intake / Weight gained

Gross Feed Conversion Efficiency (K1) % = growth / food consumed X 100

Net Feed Conversion Efficiency (K2) % = growth / food consumed-amount of faeces X 100

Feed Efficiency (FE) % = Wet weight gained/ Dry weight of feed offered X 100

Feed intake (FI) = amount of feed consumed

Protein intake (PI) = Protein present in consumed feed

Amino acid intake (AI) = Amino acid present in consumed feed

Carbohydrate intake (CI) = carbohydrate present in consumed feed

Lipid intake (LI) = lipid present in consumed feed

Protein Efficiency Ratio (PER) = Weight gained/ Protein consumed

Protein Conversion Ratio (PCR)=Protein gained/ Protein consumed

Protein Assimilation Rate (PAR) % = Protein consumed - Fecal protein / Protein consumed X 100

Protein Productive Value (PPV) % = Protein gained / Protein absorption X 100

Energy utilization analysis

Similar feeding trials were conducted with P.L. in triplicates for calculating food utilization parameters, such as feeding rate, mean absorption, mean conversion and metabolic rate. The energy content of whole prawn, feeds, faeces and exuvia was measured using Parr-1281-oxygen Bomb Calorimeter. The energy budget was calculated using the equation (C = (P+E + R + F +U) derived by (Petrusewicz and Macfadyen, 1970); where, C is the energy consumed in food; P, is the conversion or growth; R, the material lost as heat due to metabolism; F, the energy lost through faeces; U, the energy lost in ammonia excretion and; E, the energy lost through exuvia. The daily excretion of ammonia by the prawn was estimated after feeding as per the phenol hypochloride method of (Solorzano, 1969). The energy loss occurring by ammonia excretion was calculated using the ammonia calorific quotient, 1 mg NH3:5.9 cal (Elliot, 1976). The food

energy consumed was measured as the difference between the energy content of food offered and that of the uneaten food. The quantity of absorbed food energy was calculated by subtracting F from C. Conversion or growth is the sum of energy channeled to somatic growth (P) and exuvia (E). Following the estimations of C, F, U, and P, the metabolism (R=Respiration) was calculated by dividing the respective amount of energy by initial live weight of the prawn per unit time in days.

Feeding Rate (FR) = Mean Food Consumption (k.cal/g/day) / Initial live weight of the prawn (g)

Absorption Rate (AR) = Mean Absorption (k.cal/g/day) / Initial live weight of the prawn (g)

Conversion Rate (CR) = Mean Conversion (k.cal/g/day) / Initial live weight of the prawn (g)

Excretion Rate (ER) = Mean NH_3 Excretion (k.cal/g/day) / Final live weight of the prawn (g) Metabolic rate (MR) = Absorption Rate (kg.cal/g/day) – Conversion Rate (kg.cal/g/day) + NH3 excretion Rate (kg.cal/g/day)

Statistical analysis

Student t-test (Zar, 1984) was assumed in this study to compare the mean values of different parameters in controls to those of each experimental groups using S.P.S.S. software (version, 11.5) of I.B.M .Company, U.S.A.

RESULTS AND DISCUSSION

Diet plays a vital role in the production of the antioxidant defense system by providing essential nutrient antioxidants such as vitamin E, C and β - carotene, other antioxidant plant phenols including flavanoids and essential minerals like selenium, that form important antioxidant enzymes (Rao, 2003). Antioxidant compounds supplied through diet are termed as exogenous antioxidants. The tissue levels of vitamin-C (28.69±2.10) at $P < 0.025$ and vitamin-E (20.48±3.05) at $P < 0.080$, iron (1.1±0.15) at $P < 0.044$, phosphorus (3.27±0.40) at $P < 0.082$, sodium (0.21±0.03) at $P < 0.117$ and potassium (0.24±0.06) at $P < 0.129$ has been maintained by their dietary inclusions (Table 2 and Table 3). Supplementation of vitamin-E has known to improve growth, metabolism and survival of *Macrobrachium rosenbergii* post larvae (Dandapat *et al*., 2003). Sodium (0.86±0.19) at $P < 0.118$ and potassium (0.35±0.01) at $P < 0.030$ levels was found to be higher in 5% *Curcuma longa* supplemented diet (Table 2). Supplementation of sodium chloride has known to improve the growth of carp and prawn (Keshavanath *et al*.., 2003).

A main objective of culture is to maximize both survival and growth rates at the least cost. P.L. nutrition is a critical step in culturing commercially important crustaceans (Velu and Munuswamy, 2007). A diet with 'insufficient' nutrition will induce 'cannibalism' (Habashy, 2009). Survival rate (SR) was found to be higher in the animal groups fed with 5% *Curcuma longa* supplemented diet (83.66±0.47) at $P < 0.049$ level. Herbal products have the ability to improve survival rate through its antimicrobial and anti-stress properties (Citarasu *et al*., 2002). Turmeric has been reported as a cheap, commercial bioresource as fish additive in fish feed as it has been a good source of carotenoids (Mukherjee *et al*., 2009). The principle active compound in turmeric is curcumin, which is known to contain antioxidant properties (Menon and Sudheer, 2007). Flavanoids are one among the natural active principle compounds that have been reported to promote various activities like anti-stress, growth promotion, appetite stimulation, tonic and immunostimulation and to have aphrodisiac and antimicrobial properties in finfish and shrimp larviculture (Citarasu, 2009; Sivaram *et al*., 2004). Consequently the survival rate being higher in the groups fed with diet rich in flavanoid concentration (0.64±0.009 mg/g). Survival rate also increases with increasing dietary vitamin-C concentrations as previously described by Weiqing *et al.*, (2002) in shrimps (Table 2 and Table 3).

Zingiber officinale has known to improve digestive enzyme activity and could improve feed intake and production efficiencies (Venketramalingam *et al*., 2007). A higher feed conversion ratio (FCR) of the control diet (5.52±0.40) can be explained by a higher energy requirement for assimilation at higher food consumption (Table 4), which is usually accompanied by reduced nutrient absorption and growth (Rebecca and Bhavan, 2011). There is a correlation between feed efficiency (FE) (78.71±9.34) with the gross feed conversion ratio

(GFCR) (76.97±0.61) and net feed conversion ratio (NFCR) (77.65±2.80). A low calorie diet results in a high feed conversion ratio and hence, in low efficiency, implying that prawns consume more food to overcome energy insufficiency (Harper, 1976).

Nutrition is regarded as a key factor controlling survival and growth of crustacean culture (Verri *et al.,* 2001). Enhanced nutrient intake directly correlates with the weight gain (WG) (1.37±0.45) at P < 0.046, daily growth rate (DGR) (1.52±0.5) at P < 0.045 levels (Table 4) and their relative concentrations in the muscle tissue of the diet-fed prawns (Table-3). Increased intake of Proteins (PI) (196.92±1.56) at P < 0.034, carbohydrates (CI) (135.65±1.08) at P < 0.151, amino acids (AI) (65.72±0.52) at P < 0.035 and lipids (LI) (26.68±0.21) at P < 0.199 levels in the groups fed with 5% *Z. officinale* supplemented diet is a consequence of the increased feed intake (FI) 1.78±0.1 at P < 0.095 level (Table 4) and thus have contributed for energy production (Table 5). Increased intake of feed, thereby the nutrients present in it have aided for the energy requirements of the prawns. Growth and metabolism are sustained by the energy generated from the catabolism of either dietary protein or non-protein energy sources (Ashraf and Goda, 2008). Animals rely on a functional digestive system to efficiently utilize the nutrients present in the food (Anderson and De Silva, 2003). *Zingiber officinale* also appears to aid in protein absorption. The protein absorption rate is higher (P < 0.030) in the groups fed with 5% *Zingiber officinale* supplemented diet (99.04±0.01), as *Zingiber officinale* is also one of the best carrier herbs and it could help in digestive absorption by up to 200% (Belewu, 2006; Ghayur and Gilani, 2005) and the efficiency (6.81±0.16) with which the protein is converted (1.75±0.04) and produced (176.87±4.71) correlates directly with the amount of protein taken and protein absorbed. Therefore, the increase in enzyme production is considered to have resulted in improvements in digestibility and availability of nutrients from feedstuffs, resulting in increased energy production (Table 5). Dietary protein requirements are closely related to dietary energy levels, which is evident from the efficiency with which protein is converted directly correlates with the energy production.

Table 2. Concentration of minerals and non-enzymatic antioxidants in the formulated feeds.

Parameters	Feeds									
		Experimental								
	Control Feed-1	Feed -2 (1% A.S.)	Feed -3 (3% A.S.)	Feed -4 (5% A.S.)	Feed -5 (1% C.L.)	Feed -6 (3% C.L.)	Feed -7 (5% C.L.)	Feed -8 (1% Z.O.)	Feed -9 (3% Z.O.)	Feed -10 (5% Z.O.)
Iron (mg/g)	0.45 ±0.08	0.56 ±0.10	0.60 ±0.09	0.62 ±0.04	0.77 ±0.05	0.87 ±0.05	1.09 ±0.03	0.85 ±0.16	0.94 ±0.20	0.99 ±0.13
Phosphorus (mg/g)	5.29 ±0.32	9.82 ±0.27	10.09 ±0.05	12.74 ±1.00	4.70 ±0.72	5.48 ±0.31	5.79 ±0.17	6.69 ±0.14	7.16 ±0.90	7.68 ±0.88
Sodium (mg/g)	0.08 ±0.01	0.11 ±0.01	0.15 ±0.01	0.24 ±0.02	0.28 ±0.01	0.31 ±0.01	0.86 ±0.19	0.15 ±0.02	0.31 ±0.03	0.39 ±0.01
Potassium (mg/g)	0.04 ±0.01	0.05 ±0.01	0.06 ±0.01	0.28 ±0.02	0.26 ±0.01*	0.31 ±0.01	0.35 ±0.01*	0.12 ±0.01	0.13 ±0.01	0.23 ±0.01
Vitamin-C (mg/g)	7.83 ±2.09	9.44 ±0.88	9.75 ±1.36	12.05 ±0.69	10.03 ±1.45	10.77 ±1.84	12.29 ±0.15	8.62 ±3.28	9.04 ±3.42	10.44 ±2.81
Vitamin-E (mg/g)	1.01 ±0.11	2.11 ±0.74	3.42 ±0.78	5.42 ±0.29	4.21 ±0.63	5.76 ±1.28	7.47 ±0.77	8.34 ±0.81	11.13 ±0.59	15.43 ±2.93
Flavanoids (mg/g)	0.19 ±0.01	0.19 ±0.01	0.29 ±0.02	0.31 ±0.01	0.31 ±0.03	0.45 ±0.03	0.64 ±0.01	0.30 ±0.02	0.32 ±0.02	0.44 ±0.03

**Each value is mean ± SD of triplicate observations.

Significance (P<) of paired samples t-test are given in parentheses. (* the correlation and t cannot be computed because the SE of the difference is '0').

Table 3. Concentrations of biochemical constituents in P.L. of *M.rosenbergii* fed with formulated feeds.

Parameters	Initial	Groups									
			Experimental								
		Control Group-1	Group -2 (1% A.S.)	Group -3 (3% A.S.)	Group -4 (5% A.S.)	Group -5 (1% C.L.)	Group -6 (3% C.L.)	Group -7 (5% C.L.)	Group -8 (1% Z.O.)	Group -9 (3% Z.O.)	Group -10 (5% Z.O.)
Protein (%)	26.96 ±2.45	87.80 ±7.89	107.14 ±1.94	146.51 ±7.94	158.44 ±3.60	117.1 ±7.09	93.96 ±6.72	89.62 ±5.09	159.41 ±1.52	179.06 ±6.57	237.14 ±6.24
Amino acid (%)	09.10 ±1.24	44.26 ±4.42	59.36 ±3.79	64.41 ±0.72	73.57 ±2.04	54.43 ±7.40	48.05 ±0.75	40.59 ±7.09	46.30 ±3.10	59.28 ±6.72	76.82 ±2.82
Carbohydrate (%)	23.75 ±2.28	76.32 ±9.35	93.30 ±9.74	100.5 ±3.36	106.7 ±7.72	101.8 ±9.04	74.81 ±7.08	77.37 ±7.26	88.54 ±8.75	104.6 ±4.43	113.8 ±10.06
Lipids (%)	2.46 ±0.37	7.68 ±0.42	8.09 ±1.20	9.75 ±0.63	11.16 ±0.31	11.62 ±0.64	10.43 ±0.90	9.05 ±0.67	8.80 ±0.19	10.35 ±0.74	11.71 ±0.16
Moisture (%)	91.00 ±4.24	73.00 ±9.89	70.5 ±7.78	66.50 ±6.26	61.50 ±4.95	63.00 ±7.07	69.00 ±2.73	71.00 ±1.41`	65.00 ±7.07	64.50 ±6.36	59.50 ±2.12
Ash (%)	4.50 ±0.71	8.50 ±0.71	10.00 ±1.41	13.00 ±1.41*	13.50 ±0.71*	11.50 ±0.71*	13.50 ±0.71	14.00 ±1.41	11.00 ±1.41	13.00 ±1.41*	13.50 ±0.71
Iron (mg/g)	0.035 ±0.07	0.15 ±0.06	0.16 ±0.06	0..36 ±0.07	1.08 ±0.66	0.32 ±0..04	0.55 ±0.02	1.10 ±0.15	0.30 ±0.08	0.48 ±0.05	0.79 ±0.04
Phosphorus (mg/g)	0.025 ±0.07	0.88 ±0.03	1.65 ±0.18	1.99 ±0.11	3.27 ±0.40	0.89 ±0.09	1.39 ±0.28	1.88 ±0.17	1.07 ±0.06	1.74 ±0.12	1.92 ±0.11
Sodium (mg/g)	0.04 ±0.01	0.06 ±0.01	0.07 ±0.08	0.10 ±0.02	0.11 ±0.01	0.17 ±0.01	0.19 ±0.03	0.21 ±0.03	0.12 ±0.01*	0.13 ±0.01	0.14 ±0.01
Potassium (mg/g)	0.05 ±0.01	0.06 ±0.01	0.06 ±0.01	0.08 ±0.01	0.10 ±0.01	0.16 ±0.03	0.15 ±0.01	0.24 ±0.06	0.09 ±0.01	0.13 ±0.004	0.15 ±0.01
Vitamin-C (mg/g)	5.25 ±0.89	10.15 ±1.09	13.65 ±2.26	15.67 ±0.83	20.44 ±0.99	23.32 ±0.95	23.91 ±1.11	28.69 ±2.10	20.38 ±3.21	21.41 ±2.100	22.98 ±1.03
Vitamin-E (mg/g)	3.38 ±0.54	7.67 ±0.76	9.20 ±0.81	10.82 ±0.23	12.83 ±0.77	9.45 ±0.15	11.46 ±0.79	17.41 ±1.32	11.76 ±0.63	13.86 ±1.10	20.48 ±3.05

Table 4. Growth performance, survival, feed quality and nutritional indices in *M. rosenbergii* P.L. fed with formulated feeds.

Parameters	Control Group-1	Groups								
		Experimental								
		Group -2 (1% A.S.)	Group-3 (3% A.S.)	Group -4 (5% A.S.)	Group -5 (1% C.L.)	Group -6 (3% C.L.)	Group -7 (5% C.L.)	Group -8 (1% Z.O.)	Group -9 (3% Z.O.)	Group -10 (5% Z.O.)
Morphometric data and Survival										
Length (cm) Initial	2.08 ±0.28	2.08 ±0.28	2.08 ±0.28	2.08 ±0.28	2.08 ±0.28	2.08 ±0.28	2.08 ±0.28	2.08 ±0.28	2.08 ±0.28	2.08 ±0.28
Length (cm) Final	3.70 ±0.3	3.90 ±0.4	4.00 ±0.5	5.80 ±0.2	5.60 ±0.36	4.20 ±0.6	4.00 ±0.5	4.50 ±0.45	4.90 ±0.40	6.30 ±0.50
Weight (g) Initial	0.12 ±0.05	0.12 ±0.05	0.12 ±0.05	0.12 ±0.05	0.12 ±0.05	0.12 ±0.05	0.12 ±0.05	0.12 ±0.05	0.12 ±0.05	0.12 ±0.05
Weight (g) Final	0.35 ±0.04	0.38 ±0.05	0.53 ±0.04	1.00 ±0.20	0.95 ±0.05	0.62 ±0.04	0.59 ±0.09	0.83 ±0.05	0.92 ±0.04	1.47 ±0.45
WG (g)	0.25 ±0.04	0.30 ±0.02	0.43 ±0.04	0.90 ±0.20	0.85 ±0.05	0.52 ±0.04	0.49 ±0.09	0.73 ±0.05	0.82 ±0.04	1.37 ±0.45
DGR (%)	0.28 ±0.05	0.33 ±0.03	0.48 ±0.05	0.99 ±0.21	0.94 ±0.06	0.58 ±0.06	0.55 ±0.10	0.82 ±0.06	0.91 ±0.05	1.52 ±0.50
SR (%)	62.00 ±2.83	65.34 ±1.89	67.67 ±6.13	70.67 ±3.77	67.00 ±4.24	72.34 ±6.13	83.66 ±0.47	64.00 ±5.66	70.50 ±3.77	73.00 ±4.24
Feed Quality & Nutritional indices										
FI (g/g)	1.38 ±0.10	1.58 ±0.05	1.60 ±0.04	1.68 ±0.04	1.62 ±0.03	1.55 ±0.04	1.50 ±0.02	1.68 ±0.04	1.70 ±0.03	1.78 ±0.10
Fecal Output (mg/g)	39.53 ±2.38	37.19 ±2.43	27.03 ±2.93	22.13 ±3.66	37.63 ±1.67	33.88 ±3.79	24.32 ±2.99	27.03 ±2.93	23.90 ±3.22	16.82 ±1.94

D (%)	97.14 ±0.16	97.66 ±0.18	98.32 ±0.18	98.66 ±0.20	97.69 ±0.13	97.81 ±0.28	98.40 ±0.18	98.39 ±0.17	98.59 ±0.17	99.07 ±0.12
FCR	5.52 ±0.40	5.25 ±0.17	3.79 ±0.03	1.86 ±0.42	1.91 ±0.04	2.98 ±0.08	3.40 ±0.05	2.29 ±0.05	1.36 ±0.03	1.30 ±0.01
GFCR (%)	18.16 ±1.27	19.06 ±0.60	26.89 ±0.65	53.75 ±1.14	52.48 ±1.27	33.56 ±0.91	29.43 ±0.42	43.59 ±0.92	47.06 ±0.78	76.97 ±0.61
NFCR (%)	18.66 ±2.99	19.48 ±1.30	27.39 ±2.05	54.22 ±3.17	53.80 ±2.63	34.21 ±3.05	33.33 ±2.38	44.44 ±6.09	48.81 ±3.00	77.65 ±2.80
FE (%)	13.89 ±2.22	16.67 ±1.11	23.89 ±2.22	50.00 ±1.10	47.22 ±2.78	28.89 ±2.22	27.41 ±5.01	40.74 ±2.79	45.56 ±2.23	78.71 ±9.34
PER	2.23 ±0.16	2.05 ±0.06	2.84 ±0.10	4.62 ±0.10	5.65 ±0.08	3.84 ±0.11	3.25 ±0.05	4.97 ±0.11	4.66 ±0.09	6.81 ±0.16
PCR	0.25 ±0.01	0.28 ±0.07	0.49 ±0.01	0.80 ±0.04	0.72 ±0.02	0.41 ±0.01	0.34 ±.07*	0.88 ±0.02	0.92 ±0.02	1.75 ±0.04
PAR (%)	97.10 ±0.21	97.64 ±0.08	98.31 ±0.06	98.68 ±0.03	97.66 ±0.04	97.80 ±0.06	98.39 ±0.02	98.39 ±0.03	98.58 ±0.02	99.04 ±0.01
PPV (%)	25.38 ±3.17	27.83 ±0.26	27.12 ±1.53	40.47 ±0.94	74.10 ±4.62	41.81 ±3.15	33.61 ±2.07	89.32 ±0.88	93.15 ±3.54	176.87 ±4.71
	Energy intake (mg/g)									
PI	112.37 ±7.96	146.65 ±4.61	151.58 ±5.36	194.64 ±3.73	150.35 ±2.19	135.41 ±3.7	135.12 ±1.92	146.70 ±3.10	171.85 ±2.86	196.92 ±1.56
AI	39.13 ±2.81	48.13 ±1.52	52.66 ±1.90	65.11 ±1.37	48.84 ±0.90	48.14 ±1.32	49.56 ±1.07	50.64 ±1.07	54.06 ±0.91	65.72 ±0.52
CI	114.77 ±8.24	123.25 ±3.87	126.72 ±0.95	134.00 ±2.83	115.80 ±2.02	110.41 ±3.0	101.23 ±1.44	120.38 ±2.54	128.98 ±2.15	135.65 ±1.08
LI	23.47 ±1.68	20.97 ±0.66	20.87 ±0.74	16.68 ±0.35	25.66 ±0.45	24.61 ±0.67	20.57 ±0.30	26.55 ±0.57	26.42 ±0.43	26.68 ±0.21

**Each value is mean ± SD of triplicate observations

WG- Weight Gain; DGR-Daily Growth Rate; SR-Survival Rate; D-Digestibility; FCR-Food Conversion Ratio; GFCR - Gross Feed Conversion Ratio; NFCR-Net Feed Conversion Ratio; FE-Feed Efficiency; PER-Protein Efficiency Ratio; PCR-Protein Conversion Ratio; PAR-Protein Assimilation Rate; ; PPV- Protein Productive Value.

Significance (P<) of paired samples t-test are given in parentheses. (* the correlation and t cannot be computed because the SE of the difference is '0').

Table 5. Energy budgets of prawns fed with different feeds for a period of 90 days.

k.cal/ day	Groups									
		Experimental								
	Control Group-1	Group -2 (1% A.S.)	Group -3 (3% A.S.)	Group -4 (5% A.S.)	Group -5 (1% C.L.)	Group -6 (3% C.L.)	Group -7 (5% C.L.)	Group -8 (1% Z.O.)	Group -9 (3% Z.O.)	Group -10 (5% Z.O.)
FR	0.40 ±0.04	0.42 ±0.03	0.47 ±0.04*	0.68 ±0.01	0.65 ±0.01	0.54 ±0.03	0.49 ±0.02	0.59 ±0.01	0.69 ±0.04	0.85 ±0.04
AR	0.32 ±0.01	0.37 ±0.05	0.44 ±0.06	0.64 ±0.03	0.58 ±0.01*	0.46 ±0.03	0.35 ±0.07	0.64 ±0.04	0.80 ±0.01	0.90 ±0.01
CR	0.06 ±0.001	0.08 ±0.007	0.13 ±0.028	0.24 ±0.001*	0.23 ±0.008	0.14 ±0.001*	0.12 ±0.003	0.20 ±0.014	0.25 ±0.02	0.40 ±0.01
U	0.03 ±0.003	0.037 ±0.004	0.039 ±0.001	0.042 ±0.001	0.04 ±0.004	0.038 ±0.004	0.035 ±0.01	0.040 ±0.001	0.04 ±0.01	0.05 ±0.01
MR	0.32 ±0.01	0.39 ±0.03	0.40 ±0.04	0.44 ±0.04	0.40 ±0.01	0.37 ±0.04	0.35 ±0.04	0.42 ±0.04	0.44 ±0.03	0.49 ±0.01*

**Each value is mean ± SD of triplicate observations.

Significance (P<) of paired samples t-test are given in parentheses.

FR – Feeding rate, AR- Absorption rate, ECR- Energy Conversion rate, U- Ammonia excretion and MR- Metabolic rate.

(* the correlation and t cannot be computed because the SE of the difference is '0').

The rate of feeding, absorption, conversion, ammonia excretion and metabolism of the prawns fed with ten differently prepared diets were presented in the Table-5. These were found to be significantly higher in the groups of prawn fed with 5% *Zingiber officinale*. Groups fed with the control diet showed lesser values compared to the other groups. Metabolism is the set of chemical reactions that happen in living organisms to maintain life. These processes allow organisms to grow and reproduce, maintain their structures, and respond to their environments. The increase in metabolism after feeding is called specific dynamic action (Du and Niu, 2002). From this it is evident that, increased metabolic rates in the groups fed with *Z. officinale* favored better growth of the prawns. Groups fed with *Allium sativum* have also shown to increase energy production, which increased with increased supplementation. However, the production increased initially with the supplementation of *Curcuma longa* and then reduced with its increased supplementation.

The present study clearly indicates that the protein requirements of the prawn have been met especially when these are fed with the diet supplemented with *Z. officinale* which could improve feed and nutrients intake as the excess protein in the diet will be metabolized by prawns as a source of energy, and nitrogen will be excreted as ammonia. Recently ammonia and other environmental parameters have known to affect immune system of fishes, molluscs and shrimps (Verghese *et al*., 2007). Ammonia is toxic to fish (Drahos, 2007). However in the present study, the concentration of ammonia in the water medium does not correlate with the survival rate of the freshwater prawns. Groups fed with *C. longa* attained maximum survival rate compared to the other groups (Table 4) in spite of its increased ammonia excretion rate compared to control (Table-5). It is ascertained that the group fed with *C. longa* attained ammonia tolerating feature by the presence of antioxidant defense system (AD) comprising of non-enzymatic (vitamin-C and vitamin-E) and enzymatic antioxidants (SOD, CAT and GPX) as studied by Mitra *et al*. (2005). However in the water medium the concentration of ammonia is affected by the rate of feed intake (Gonzalez-Pena and Moreira, 2003). Ammonia in the water medium is the result of fish waste and decomposing food in the aquarium. Increased feed intake by the group fed with 5% *Z. officinale* (Table 4) does not correlate with survival rate of the prawns, ascertaining that, the presence of antioxidant defense system (AD), provided for improved survival of the freshwater prawns.

CONCLUSIONS

This preliminary investigation has demonstrated that the selected herbs when supplemented at suitable proportion, have promoted the growth and survival of *M. rosenbergii* post larvae. Herb supplemented diets not only provided essential vitamins and minerals for P.L., but also met the energy requirements, hence it does not appear to contribute for any adverse effects on P.L. owing to its use. The growth promoting property is attributed to *Z. officinale* and the survival enhancement property is attributed to *C. longa*. *A. sativum* moderately possess these two properties. Therefore, it is suggested that these herbs can be used as cheap and safer alternatives against synthetic hormones and antibiotics for its growth and survival.

CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interests associated with this article.

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