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Influence of medicinal herbs, *Andrographis paniculata*, *Cissus quadrangularis* and *Eclipta alba*) on growth, digestive enzymes, biochemical constituents and protein profile of the freshwater prawn *Macrobrachium rosenbergii*

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ABSTRACT

This study was conducted to understand the potential of medicinal herbs, *Andrographis paniculata*, *Cissus quadrangularis* and *Eclipta alba* on growth promotion, utilization of energy, induction in activities of digestive enzymes, concentrations biochemical constituents and non-enzymatic antioxidants of the freshwater prawn *Macrobrachium rosenbergii*. These herbs were incorporated with basal diets at three different concentrations (1%, 3%, and 5%) individually and fed to the post larval (PL) of *M. rosenbergii* (1.83±0.50 cm; 0.14± 0.02 g) for a period of 90 days in a triplicate experimental set-up. A significantly improved survival and growth performance (weight gain, specific growth rate, condition factor), elevation in energy utilization, increased activities of digestive enzymes (protease, amylase and lipase), concentrations of biochemical constituents (total protein, carbohydrate and lipid) and non-enzymatic antioxidants (vitamin-C and vitamin-E) were recorded ($P<0.05$) in *C. quadrangularis* incorporated feed fed PL (5%>3%>1%), followed by *E. alba* (5%>3%>1%) and *A. paniculata* (3%>5%>1%) when compared with control diet (without incorporation of any herb) fed PL. Twelve polypeptide bands of molecular weight between 116-14 were recorded in the muscle of PL fed with control as well as the best concentration of each herb incorporated feeds. The intensity of 50, 48, and 46 kDa regions was found to be stained more in *C. quadrangularis* incorporated feed fed PL when compared with control feed as well as *E. alba* and *A. paniculata* incorporated feeds fed PL. In this study, the overall results indicated the fact that these herbs enhanced the secretion of digestive enzymes, act as appetizer, which facilitate efficient digestion, absorption of nutrients and favour general metabolism, which in turn ultimately improved the general health and produced better survival and growth of *M. rosenbergii* PL. Therefore, it is suggested that these herbs have the characteristic ability to promote growth in *M. rosenbergii*. The aquaculture potential of these herbs needs further study based on their active principles.

Introduction

World aquaculture industry witnessed a phenomenal growth by way of increment in production from 24.38 million tonnes in 1995 to more than double in the year 2006, 51.70 million tones, and India with its aquaculture production of 3.12 million tones is placed second only after China (FAO, 2009). Freshwater crustaceans including prawns though produced in meager quantities (around 953,198 tonnes) have gained significance as high value commodity by standing as fifth highest valued species group. One of the major prawn species now dominating the freshwater aquaculture in India is the giant freshwater prawn (scampi), *Macrobrachium rosenbergii*, and it is one of the major contributors of national economy as well (Akand and Hasan, 1992; Ahmed, 2001; Muir, 2003). The production of *M. rosenbergii* in India increased from 7150 tonnes during the year 1999-2000 to 27, 262 tonnes in 2007-2008 (MPEDA, 2008). Farming of *M. rosenbergii* is spreading fast to all Indian states due to its large size attainment, tolerance to water quality changes, ability to cope with handling stress and ability to feed on unconventional feeds. The farming of scampi received a big boost lately owing to the demand and attractive price fetched in the international market and the slump in tiger shrimp production due to outbreak of disease.

There is a developing interest in using medicinal herbs as a kind of immune stimulant in aquaculture (Luo, 1997; Chansue *et al.*, 2000). Administration of medicinal herbs with shrimp diet increases the specific activity of amylase and protease in the shrimp's digestive gland and improves growth performance. Medicinal herbs increased apparent digestibility of lipid and phosphorus, but decreased apparent digestibility of crude protein (Lin *et al.*, 2006). Previous studies on *Macrobrachium* revealed that feeds formulated with cereals, pulses, groundnut oilcake, feeds incorporated with vegetable wastes, and herbals have resulted in appreciable growth and sustainability (Bhavan *et al.*, 2010, 2011a, 2011b; Rebecca and Bhavan *et al.*, 2011).Therefore few locally available medicinal herbs, *Andrographis paniculata*, *Cissus quadrangularis* and *Eclipta alba* were chosen based on their active principles reported to demonstrate whether these herbs have growth promoting ability on *M. rosenbergii* PL.

A. paniculata (Nilavembu) is one of the most important medicinal herbs and having been widely used in Ayurvedic medicine for the treatment of gastric disorders, infectious

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diseases and common cold. *A. paniculata* possesses anti-inflammatory, antiallergenic, immune–stimulatory, antiviral, antioxidant, hepatoprotective, cardiovascular activities. Diterpenoids and flavonoids are the main chemical constituents of *A. paniculata,* and these compounds are believed to be responsible for the biological activities of the plants (Tang *et al.*, 1992).

C. quadrangularis (Pirandai) is one of the traditional recipes for treatment of physical and mental ailments exists in all major ancient civilizations of the world (Kumbhojkar *et al*., 1991). This plant is routinely used to accelerate the process of bone fracture healing (Udupa *et al*., 1964). Various organic macromolecules ranging from terpenoids to large stilbene derivatives have been isolated from this plant (Adesanya *et al.*, 1999). Presence of tetracyclic triterpenoids, β-sitosterol, δ-amyrin, isopentacosanoic acid, flavonoids such as quercetin, kaempferol, steroidal principles, β-carotene, and vitamin C in this plant have been reported (Swamy *et al.*, 2010). Stem and root extract of this plant possess antimicrobial activity against Gram-positive bacteria, including *Bacillus subtilis, Bacillus cereus, Staphylococcus aureus,* and *Streptococcus* species (Chidambara, 2003; Murthy *et al.*, 2003).

E. alba (karishalanganni) is a well known herb in the Indian system of medicine. It is applied externally as antiseptic to tonic, spasmogenic, ovicidal in ulcers and wounds. Also used as healing of fractures. *E. alba* is possesses hepatoprotective property (Kaur *et al.*, 2009). It has active principles of phenolic compounds, which are thought to be responsible for antioxidant activity. The extracts are powerful scavengers of free radicals and nitric oxide radicals, as well as efficient inhibitors of lipid peroxidation (Kaur *et al.*, 2009).

In this study, influence of these herbs on survival and growth promotion, utilization of energy, induction in activities of digestive enzymes (protease, amylase and lipase), concentrations of biochemical constituents (total protein, carbohydrate and lipid), levels of non-enzymatic antioxidants (vitamin-C and vitamin-E), and pattern of protein profile of the freshwater prawn *M. rosenbergii* were studied by feeding this prawn with these herbs incorporated feeds.

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. At the time of procurement the hatchery water had these physic-chemical characteristics: 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. They were transported to the laboratory in oxygenated polythene bags and acclimatized for two weeks (up to reaching 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). The prawns were fed with boiled egg albumin, *Artemia* nauplii and commercially available scampi feed alternatively thrice a day. 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 40% protein were formulated by 'Pearson square' method using the following basal ingredients, such as fishmeal (15%), soy meal (15%), corn flour (12%), wheat bran (12%), rice bran (12%) and groundnut oil cake (20%) in powder form. Tapioca flour (7%) 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. Cod-liver oil (1 ml) was added as lipid source. Vitamin B-complex with vitamin-C (1 %) was also mixed. With this, each herbal powder (*A. paniculata, C. quadrangularis* and *E. alba*) was separately added in three different concentrations (1g, 3g, and 5g/ 100 g) and mixed well. The feed ingredients were chosen on the basis of their nutritional status, price and year round availability in the local market. Actually, medicinal herbs were homogenized with 10 ml distilled water for 10 minutes to bring the final mixture into a paste form. 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 stacked in plastic containers and kept in a cool, dry place and fed to *M. rosenbergii* PL.

The experiment was conducted in triplicate with 25 animals each (PL-30; length: 1.83±0.50 cm; weight: 0.14± 0.02 g) in 30 plastic aquaria of 25 liters capacity (each herb represent three diets, and a common control). Each group was fed with a specific diet formulated *ad libitum* (1g per aquarium) for a period of 90 days. The water medium was renewed daily by siphoning method without severe disturbance to the prawn. The faecal matter, unfed feed, and exuvia if any were removed. The medium was aeration. 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 by using following formulae.

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

$$\text{Weight Gain (WG)} = \text{Final weight (g)} - \text{Initial weight (g)}$$

$$\text{Biomass Index (BI)} = \frac{\text{Final weight (g)} - \text{Initial weight (g)}}{\text{Initial weight (g)}} \times 100$$

$$\text{Specific Growth Rate (SGR)} = \frac{\log \text{ of Final weight (g)} - \log \text{ of Initial weight (g)}}{\text{No. of days}} \times 100$$

$$\text{Condition Factor (CF)} = \frac{\text{Final weight (g)}}{\text{Final length}^3 \text{ (cm)}} \times 100$$

The energy content of whole prawns, feeds, moult and faeces was measured using Parr 1281 Oxygen Bomb Calorimeter. The energy utilization 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 growth; R is the material lost as heat due to metabolism; F is the energy lost in faeces; U is the energy lost in excretion and; E is the energy lost in exuvia.

$$\text{Feeding Rate (FR)} = \frac{\text{Mean Food Consumption (k.cal./day)}}{\text{Initial live weight of the prawn (g)}}$$

$$\text{Mean Absorption} = \text{Mean Food Consumption (k.cal./day)} - \text{Mean Food Excreted as Faeces (k.cal/day)}$$

$$\text{Absorption Rate (AR)} = \frac{\text{Mean Absorption (k.cal/day)}}{\text{Initial live weight of the prawn (g)}}$$

$$\text{Mean Conversion} = \text{Mean weight gain (k.cal./day)} + \text{Mean exuvial weight (k.cal/day)}$$

$$\text{Conversion rate (CR)} = \frac{\text{Mean Conversion (k.cal./day)}}{\text{Initial live weight of the prawn (g)}}$$

$$NH_3\text{ExcretionRate AE)} = \frac{\text{Mean } NH_3 \text{ Excretion (k.cal./day) } NH_3}{\text{Initial live weight of the prawn (g)}}$$

Metabolic Rate (MR) = Absorption Rate (k.cal./g/day) – Conversion Rate (k.cal/g/day) + NH_3 excretion Rate (k.cal./g/day)

The digestive enzymes activities, such as protease, amylase and lipase were determined by the method of Sarath *et al.*, (1989), Bernfeld (1955) and Albro *et al.*, (1985) respectively. Concentrations of total protein, carbohydrate, and lipid were estimated by following the method of Lowery *et al.*, (1951), Roe (1955) and Folch *et al.*, (1957) respectively. Concentrations of non-enzymatic antioxidant, such as vitamin-C and vitamin-E were determined using the method of Roe and Kuether (1943) and Baker *et al.*, (1980) respectively. Among the three concentrations of each herb, PL showed the best overall performance was alone subjected to protein profile analysis. SDS-PAGE was performed on vertical slab gel with 4% stacking gels and 10% separating gels according to the method of Laemmli (1970). 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 (45kDa), carbonic anhydrase (29 kDa), soyabean trypsin inhibitor (20 kDa) and lysozyme (14 kDa) was also run. The patterns were compared by using information on apparent molecular masses of bands and their intensity. The data were subjected to analyses by adopting student t-test through SPSS software, version 11.5.

Results and Discussion

Nutritional indices and energy utilization

The nutritional indices, such as weight gain including biomass index, specific growth rate, condition factor and survival rate were all given in table-1. Values of these parameters except condition factor were found to be significantly increased ($P<0.05$) in experimental feeds fed PL groups when compared with control. In the case of condition factor, just the reverse was naturally recorded ($P<0.05$). Among the three herbs used, *C. quadrangularis* incorporated feed fed PL showed the best performance (5% incorporation >3% >1%), followed by *E. alba* (5% incorporation >3% >1%) and *A. paniculata* (3% incorporation >5% >1%). In the present study, the energy utilization of *M. rosenbergii* offered with these three herbs showed that there were significant increases in the feeding rate, absorption rate, conversion rate, NH_3 execratory rate and metabolic rate. Among three herbs used, *C. quadrangularis* incorporated feed fed PL showed the best performance followed by *E. alba* and *A. paniculata* in the above mentioned trend when compared with control ($P<0.05$).Therefore, 5% of *C. quadrangularis*, 5% of *E. alba* and 3% of *A. paniculata* were found to be the best concentrations of respective herbs on parameters of nutritional indices and energy utilization studied.

The increase in nutritional indices has also been reported in the following studies. In *M. rosenbergii* fed with 'Nutripro-aqua', the herbal based diet (Kesavanth and Jeyaram, 1998). In *Penaeus indicus* fed with 'stressol-I' and 'stressol-II', the commercially available herbal products enriched *Artemia* (Chitra, 1995) and 'Livol IHF-1000' a diet containing *Boerhavia diffusa, Solanum nigrum* and *Terminalia arjuna* (Sambhu and Jayaprakas, 2001). In *Penaus monodon* fed with *Withania somnifera* and *Mucuna pruriens* extracts enriched *Artemia* (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' containing *Tephrosia purpurea, Eclipta alba, Phyllanthus niruri, A. paniculata, Ocimum sanctum* and *Terminalia chebulam* enriched *Artemia* (Citarasu, 2009).

In this study, the palatability of herbal incorporated feeds may be enhanced due to their active principles. Therefore, feeding rate was higher in experimental PL groups. Feeding and absorption are the most important factors that affect growth as well as the yield, feed conversion and carcass composition of the prawn (Gupta *et al.*, 2007). Herbal influences in food utilization, such as feeding, absorption and food conversion has been reported in *P. monodon* fed with *H. spinosa, W. somnifera, Z. officinalis, S. trilobatum and A. Paniculata* and *Z. officinalis* enriched *Artemia* (Citarasu *et al.*, 2002; Venketramalingam *et al.*, 2007) and in *Labeo rohita* fed with *Sesuvium portulacastrum* supplemented diet (Johnson and Banerji, 2007). In the present study the recorded increase in ammonia excretion and metabolic rates can be correlated with increased food consumption, absorption of nutrients and ultimately reflected on growth of *M. rosenbergii* PL due to influences of *C. quadrangularis, E. alba* and *A. paniculata*.

Digestive enzymes

The activities of digestive enzymes, such as protease, amylase and lipase were 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 used, *C. quadrangularis* incorporated feed fed PL showed the most increased activity (5% incorporation >3% >1%), followed by *E. alba* (5% incorporation >3% >1%) and *A. paniculata* (3% incorporation >5% >1%). Therefore, 5% of *C. quadrangularis*, 5% of *E. alba* and 3% of *A. paniculata* were found to be the most effective concentrations of respective herbs in enhancing the activities of these digestive enzymes.

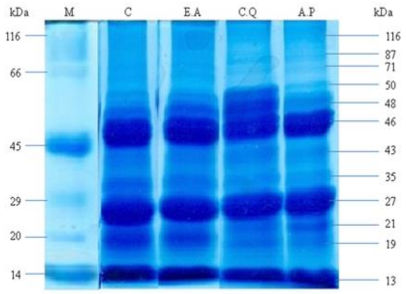
The increased activities of digestive enzymes indicated the fact that they increased appetite, which in turn enhanced food consumption and facilitated effective digestion, absorption of nutrients, and enhanced metabolism, which ultimately reflected on food conversion/growth of PL. The effect of herbal nutrients on growth has been revealed in literatures. 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 (Maheshappa, 1993; Shadakshari, 1993; Unnikrishnan, 1995; Bolle *et al.*, 1996; Jayaprakas and Euphrasia, 1996) and *P. indicus* PL (Shambu and Jayaprakas, 2001). *Z. officinalis* enriched *Artemia* was reported 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 cellulose activities in the mid-gut gland, intestine, and stomach of *M. rosenbergii* were much higher than those found in the marine species, *P. monodon*, *Penaeus japonicus*, and *Penaeus penicillatus* (Chuang *et al.*, 1985).

Biochemical concentrations and protein profile

The concentrations of biochemical constituents were also given in table-1. Concentrations of total protein, carbohydrate and lipid were found to be significantly increased (P<0.05) in experimental feeds fed PL groups when compared with control. Among three herbs used, *C. quadrangularis* incorporated feed fed PL showed the most increased concentrations of these biochemical constituents (5% incorporation >3% >1%), followed by *E. alba* (5% incorporation >3% >1%) and *A. paniculata* (3% incorporation >5% >1%). Therefore, 5% of *C. quadrangularis*, 5% of *E. alba* and 3% of *A. paniculata* were found to be the most effective concentrations of respective herbs in enhancing the contents of these basic biochemical constituents.

The polypeptide bands of molecular weight between 116-14 were recorded in the muscle of PL fed with control as well as herbs (3% of *A. paniculata*, 5% of *C. quadrangularis*, and 5% of *E. alba*) incorporated feeds (Fig.1). There were 12 coomassie blue stained protein bands, such as 116, 87, 71, 50, 48, 46, 43, 35, 27, 21, 19 and 13 kDa were identified in both control and experimental feed fed PL. Generally, the staining intensity of 50, 48, and 46 kDa regions was found to be higher in *C. quadrangularis* incorporated feed fed PL when compared with control feed as well as *E. alba* and *A. paniculata* incorporated feeds fed PL. Actually, the staining intensity of these region among three experimental diets was in the order of *C. quadrangularis* > *E. alba* > *A. paniculata*. Another notable change was seen in 21 and 19 kDa low molecular weight region. This region was stained less intensely in experimental feeds fed PL. This was in the order of *C. quadrangularis* < *A. paniculata* < *E. alba*. Therefore, herbs may facilitate utilization of certain polypeptides, and strengthen others due to integration/ elongation/ over expression.

Figure -1. SDS-PAGE pattern of proteins in the muscle of PL fed with herbs incorporated feeds



M, marker; C, control; A.P, *A. paniculata*; C.Q, *C. quadrangularis*, E.A, *E. alba*

The supplementation of the herbal principles successfully regulated the utilization of carbohydrate and lipid, which is evident from their contents and activities of digestive enzymes in experimental PL, and thereby enhances sparing of protein towards growth rather than maintenance. Further, the herbal principles may enhance protein synthesis. The herb, *S. portulacastrum* supplemented diet improved protein synthesis in *L. rohita* (Johnson and Banerji, 2007). It has been reported that certain herbs, *Massa medicata*, *Crataegi fructus*, *Artemisia capillaries* and *Cnidium officinale* promoted cellular lipid and fatty acid utilization, which facilitates protein accumulation and good growth in *Pagrus major* (Ji *et al.*, 2007). *W. somnifera* fed spawners showed higher protein values in the haemolymph that positively regulated the larval quality in *P. monodon* (Babu *et al.*, 2008). It has been reported that the herbal growth promoters helped to induce the transcription, leading to increased RNA, which coupled with increased amino acid and finally enhances protein synthesis (Citarasu, 2009).

Non-enzymatic antioxidant

The concentrations of vitamin-C and vitamin-E were also given in table-1. Concentrations of these non-enzymatic antioxidants were found to be significantly increased (P<0.05) in tissues of experimental feeds fed PL groups when compared with control. Generally, among the two tissues taken, the hepatopancreas showed higher amount of these non-enzymatic antioxidants than that of the muscle. Among three herbs used, *C. quadrangularis* incorporated feed fed PL showed maximum elevation (5% incorporation >3% >1%), followed by *E. alba* (5% incorporation >3% >1%) and *A. paniculata* (3% incorporation >5% >1%). Therefore, 5% of *C. quadrangularis*, 5% of *E. alba* and 3% of *A. paniculata* were found to be the most effective concentrations of respective herbs in enhancing the contents of these vitamins.

The increased contents of these vitamins clearly indicated the fact that the general health was improved in PL due to medicinal herbs incorporated diets. Ascorbic acid is a potent antioxidant, which scavenges reactive radicals such as hydroxyl, perhydroxyl, peroxyl and nitric oxide (Halliwell and Gutteridge, 2001). Therefore, it serves as an important protective measure against free radicals (Bendich*et al.*, 1986; Karakoe *et al.*, 1997). Ascorbic acid is believed to regenerate vitamin E from its oxidized form (Wells *et al.*, 1992) thereby raises the antioxidant status. It has been reported in fish, *Epinephelus tauvina* fed with diets containing herbal extracts, such as *Viscum album*, *Urtica dioica*, and *Z. officinalis* improved the non-specific defense mechanisms, including extracellular and intracellular respiratory burst activities, phagocytosis in blood leukocytes, total plasma protein level, specific growth rate, and condition factor (Punitha *et al*, 2008). Immanuel *et al*, (2004) reported that herbal and seaweed can be effectively used as dietary source for enhancing disease resistance as well as survival and growth promotion in *P. indicus*.

Conclusion

The enhanced absorption of nutrients due to the efficient digestive process has improved the general metabolism, which in turn increased the contents of total protein, lipid and carbohydrate in experimental PL. This was associated with appreciable levels of vitamin-C and E, promotes general health and therefore, better growth was achieved in *C. quadrangularis*, *A. paniculata*, *E. alba* incorporated feeds fed *M. rosenbergii*. This study offers promising possibilities of using these herbs to increase survival, biochemical constituents and growth promotion, however, needs further clarifications in the line of herbal active principles.

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Table 1: Nutritional indices, activities of digestive enzymes, concentrations of non-enzymatic antioxidants and biochemical constituents in *M. rosenbergii* PL fed with medicinal herbs incorporated diets

Parameters			Control diet	Experimental diets								
				A. paniculata			*C. quadrangularis*			*E. alba*		
				1%	3%	5%	1%	3%	5%	1%	3%	5%
Nutritional indices	Initial weight (g)		0.14± 0.02	0.14±0.02	0.14±0.02	0.14±0.02	0.14±0.02	0.14±0.02	0.14±0.02	0.14±0.02	0.14±0.02	0.14±0.02
	Final weight (g)		0.67±0.61	0.71±0.08	0.88±0.09	0.83±0.08	0.81±0.08	0.89±0.09	1.13±0.23	0.77±0.07	0.84±0.09	0.97±0.08
	Weight gain (g)		0.53± 0.05	0.57±0.04	0.74±0.06	0.69±0.05	0.67±0.09	0.75±0.07	0.99±0.10	0.63±0.08	0.70±0.05	0.83±0.08
	Biomass index (%)		378±3.11	407±4.50	528±2.75	492±2.05	478±5.12	535±3.73	707±4.20	450±3.38	492±2.52	592±2.95
	Specific growth rate %		0.58±0.04	0.63±0.05	0.82±0.07	0.76±0.06	0.74±0.08	0.83±0.09	1.10±0.51	0.70±0.10	0.74±0.11	0.92±0.16
	Condition factor (%)		0.95±0.09	0.85±0.07	0.79±0.06	0.81±0.08	0.84±0.07	0.86±0.05	0.82±0.06	0.89±0.08	0.87±0.06	0.80±0.05
	Survival rate (%)		74.00±4.24	75.00±4.10	92.00±5.00	79.00±4.75	88.00±4.80	90.00±5.00	96.00±5.23	80.00±4.25	84.00±4.40	94.00±5.20
Energy utilization (k.cal./g/day)	Feeding rate		0.398±0.042	0.415±0.049	0.434±0.052	0.423±0.121	0.451±0.251	0.492±0.235	0.517±0.061	0.425±0.234	0.452±0.254	0.479±0.058
	Absorption rate		0.279±0.023	0.343±0.039	0.409±0.034	0.385±0.133	0.392±0.106	0.432±0.210	0.457±0.047	0.372±0.120	0.415±0.223	0.424±0.039
	Conversion rate		0.219±0.012	0.269±0.018	0.327±0.021	0.294±0.092	0.328±0.159	0.351±0.162	0.365±0.034	0.300±0.150	0.328±0.123	0.334±0.026
	Ammonia excretion rate		0.018±0.001	0.018±0.050	0.023±0.005	0.020±0.005	0.020±0.005	0.022±0.005	0.031±0.008	0.020±0.001	0.022±0.002	0.024±0.003
	Metabolic rate		0.078±0.006	0.092±0.021	0.109±0.020	0.100±0.005	0.084±0.006	0.103±0.009	0.123±0.013	0.092±0.006	0.106±0.050	0.110±0.002
Digestive enzymes (Unit/ mg protein)	Protease		0.38±0.02	0.59±0.04	0.65±0.06	0.61±0.03	0.60±0.04	0.63±0.02	0.89±0.10	0.68±0.06	0.64±0.05	0.84±0.08
	Amylase		0.33±0.01	0.44±0.03	0.51±0.05	0.45±0.02	0.46±0.04	0.48±0.07	0.60±0.07	0.52±0.03	0.50±0.06	0.58±0.05
	Lipase (Unit X 10^3)		0.63±0.07	2.43±0.21	2.53±0.35	2.45±0.45	2.43±0.17	2.47±0.38	3.54±0.49	2.48±0.27	2.51±0.42	3.43±0.65
Biochemical constituents (mg/ g wet tissue)	Total protein		44.5 ± 2.2	51.6 ± 2.5	62.0 ± 3.0	55.6 ± 2.7	66.0 ± 3.2	69.6 ± 3.5	79.6 ± 4.2	57.8 ± 3.6	65.4 ± 3.4	74.5 ± 4.5
	Total carbohydrate		10.65 ± 0.90	14.45 ± 1.05	16.3 ± 1.10	14.8 ± 2.00	16.9 ± 1.25	17.45 ± 1.30	20.1 ± 1.65	15.35 ± 1.35	17.2 ± 1.55	19.45 ± 1.70
	Total lipid		1.35 ± 0.12	2.25 ± 0.15	2.81 ± 0.19	2.53 ± 0.23	2.99 ± 0.29	3.09 ± 0.20	3.25 ± 0.26	2.78 ± 0.19	2.85 ± 0.25	3.19 ± 0.31
Non -enzymatic antioxidants (µmol./ mg protein)	Vitamin-C	HP	120.5±5.90	123.8±6.0	126.7±6.7	124.9±6.30	125.4±6.20	126.9±6.50	127.4±6.80	121.9±6.00	122.8±6.40	124.6±6.60
		M	70.8±3.80	72.1±3.10	75.1±4.2	74.9±3.90	74.3±3.80	75.0±4.00	77.9±4.50	73.8±4.10	73.0±3.90	75.9±4.10
	Vitamin-E	HP	49.2±3.00	52.8±3.20	54.7±3.1	53.3±3.40	50.4±2.90	53.4±3.00	56.3±3.30	50.9±2.90	54.3±3.00	55.0±3.30
		M	12.1±1.20	14.2±1.50	14.9±1.4	14.5±1.40	14.6±1.42	15.0±1.40	15.6±1.60	13.8±1.00	14.4±1.20	15.1±1.50

Each value is a mean ± SD of three individual observations.
The differences recorded between control and experiments are significant ($P < 0.05$).