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Effects of spices, *Papaver somniferum*, *Elettaria cardamomum*, *Foeniculum vulgare* and *Syzygium aromaticum* on growth promotion in *Macrobrachium malcolmsonii* early juveniles

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ABSTRACT

A 45 days feeding trial was conducted on Macrobrachium malcolmsonii (2.28±0.08 cm and 0.11±0.04 g) fed with seeds of Papaver somniferum (poppy), Elettaria cardamomum (cardamom) and Foeniculum vulgare (fennel), and flower buds of Syzygium aromaticum (clove) incorporated (1%, 3% and 5% with basal diet) feeds. Feed without inclusion of any spice was served as control. These spices were acted as appetizer and enhanced the activities of protease, amylase and lipase in test prawns (P. somniferum, 1% > E. cardamomum, 1% > F. vulgare, 2% > S. aromaticum, 1%) when compared with control (P < 0.05). This suggests enhanced food consumption and assimilation, which leads to elevation of total protein, carbohydrate and lipid. The levels of vitamin C and E, sodium, potassium, and total haemocyte population were also found to be significantly elevated in these spices incorporated feeds fed prawns when compared with control (P < 0.05). The process of lipid peroxidation was decreased significantly in these spices incorporated feeds fed prawns when compared with control (P < 0.05). Therefore, activities of super oxide dismutase and catalase were not altered significantly. This suggests that incorporation of spices have reduced free radical generation. This state indicates the fact that these spices are non-toxic at test concentrations. The overall result ensures prevalence of good general health, which ultimately favoured for better survival, food conversion and growth of M. malcolmsonii.

Key words: *Prawn, Poppy, Cardamom, Fennel, Clove, Survival, Growth.*

INTRODUCTION

The commercial culture of prawns is one of the fastest growing food sectors[1-3]. In South India, freshwater prawn species particularly, *Macrobrachium rosenbergii* and *Macrobrachium malcolmsonii* are potentially important for aquaculture[4-6]. *M. malcolmsonii* (the monsoon river prawn) is the second-largest freshwater prawn next to the giant river prawn, *M. rosenbergii*[6,7]. Herbal biomedicines have the characteristic ability of growth promotion, tonic to improve the immune system, anti-microbial capability, stimulating appetite and anti-stress characteristics in aquatic animals due to their active principles, such as alkaloids, flavanoids, pigments, phenolics, terpenoids, starch, steroids and essential oils. This eco-friendly practice will not leave any side effects on the environment and reduces the problem occur due to application of synthetic hormones, antibiotics, vitamins and several other chemicals, and greatly reduces the feed cost as well[8,9]. It has been reported that incorporation of medicinal herbs, such as *Ocimum sanctum*, *Withania somnifera*, *Andrographis paniculata*, *Cissus quadrangularis*, *Eclipta alba*, *Allium sativum*, *Zingiber officinale* (Ginger as well as dried zinger), *Curcuma longa*, *Trigonella foenum-graecum*, *Murraya koenigii*, *Coriandrum sativum*, *Menthe arvensis*, *Alternanthera sessilis*, *Piper longum*, *Piper nigram*, *Myristica fragrans*, *Glycyrrhiza glabra* and *Quercus infectoria* with artificial feeds enhance the survival and growth of prawns[10-16].

Papaver somniferum (opium poppy/ kasa kasa) contains 2196 kJ (525 k.cal) energy/ 100 g seeds (carbohydrate, 28.1 g; protein, 17.9 g; fat, 41.5 g; dietary fibre, 19.5 g). Its oil contains saturated, monounsaturated and polyunsaturated fatty acids[17]. *P. somniferum* contains many volatile compounds, 2-pentylfuran, 1-pentanol, 1-hexanol and caproic acid[18,19]. It contains alkaloids, such as morphine, heroin, thebaine, codeine, papaverine, noscapine and oripavine having narcotic properties[20-22]. Its opioid possessed analgesic, antitussive, anti diarrheal, antihypertensive, anxiolytic, antidepressant, sedative and hypnotic properties. It is used as one of the culinary spices in Indian cooking.

Elettaria cardamomum (true cardamom/ green cardamom/ elakkay) contains 1303 kJ (311 k.cal) energy/ 100 g seeds (carbohydrate, 68.4 g; protein, 10.7 g; fat, 6.7 g; dietary fibre, 28 g). It has palmitic, oleic and linoleic acids. The essential oil of *E. cardamomum* contains α-terpineol, α-terpineol acetate, terpinolene, terpinene, p-cymene, terpinen-4-oil, citronellol, nerol, geraniol, methyl eugenol, trans-nerolidol, myrcene, limonene, menthone, β-phellandrene, 1,8-cineol, sabinene, heptane, α-terpenyl acetate, linalyl acetate, linalool, sabinene, limonene, borneol, β-pinene, α-pinene and humulene[23-26]. It is used to treat constipation, dysentery, digestion problems, throat troubles, infections in teeth and gums, congestion of the lungs and pulmonary tuberculosis. It has anti-cancer, anti-inflammatory, anti-microbial and analgesic activity.

Foeniculum vulgare (fennel) contains 130 kJ (31 k.cal) energy/ 100 g seeds (carbohydrate, 7.2 g; protein, 1.2 g; fat, 0.2 g; dietary fibre, 3.1 g). It is a highly aromatic and flavorful spice. It is one of the culinary spices in Indian cooking. Its aromatic essential oil contains anethole (acts as phyto oestrogens), estragole, α-fenchone (fenchyl acetate), α-pinene, champene, β-pinene, β-myrcene, thujene, limonene, δ-carene, champor, sabinene, α-phellandrene, o-cymene, eucalyptol, ɤ-terpinene, linalool, cumic aldehyde, and *p*-anisaldehyde[27-29]. It is used to treat flatulence by encouraging the expulsion of intestinal gas. It is also used for ailing various digestive problems including heartburn, bloating, loss of appetite, colic in infants and upper respiratory tract infections, coughs and bronchitis[30,31]. Fennel is used against dysmenorrhea and hypertension[32,33].

Syzygium aromaticum (clove/ kirambu) contains 197 kJ (47 k.cal)/ 100 g flower buds (carbohydrate, 10.5 g; protein, 3.2 g; fat, 0.15 g; dietary fibre, 5.4 g). The essential oil of *S. aromaticum* contains eugenol (gives pleasant and sweet aromatic fragrance), acetyl eugenol, β-caryophyllene, vanillin and crategolic acid, tannins (pain killers, like gallotannic acid and methyl salicylate), flavonoids (eugenin, kaempferol, rhamnetin and eugenitin), triterpenoids (oleanolic acid, stigmasterol and campesterol) and several sesquiterpenes (anti carcinogenic agents)[34,35]. Its active principle increase digestion by increasing gastro-intestinal enzyme secretions, and helps to relieve indigestion and constipation. It has antioxidant, anti-inflammatory, anti-flatulent, anti-parasitic and antiseptic properties. It is used as local anesthetic in dentistry against teeth and gum diseases. It is also used against asthma and allergic disorders.

Apart from their nutritional values and active principles, these spices contain vitamins, such as folates, niacin, pantothenic acid, pyridoxine, riboflavin, thiamin, vitamin C and E (particularly clove have vitamins A, C, E and K), phyto nutrients, such as carotene-ß and lutein-zeaxanthin, electrolites (Na and K), and minerals (calcium, phosphorus, magnesium, selenium, iron, zinc, manganese and copper). As these spices are known to have anti-oxidant, disease preventive and health promoting properties, the present study was primarily aimed to identify the right quantity of each spice required for incorporation with aqua feed. This was further to understand their positive effects on survival, growth, activities of digestive enzymes (protease, amylase and lipase) and contents of basic biochemical constituents (total protein, total carbohydrate and total lipid) in the early juveniles of *M. malcolmsonii*. Furthermore, in order to understand the health status of these spices incorporated feeds fed *M. malcolmsonii*, the total haemocyte count, status of non-enzymatic antioxidants (vitamin C and E) and enzymatic antioxidants (superoxide dismutase and catalase), contents of minerals (Na and K) and the quantum of lipid peroxidation (LPO) were also assessed.

MATERIALS AND METHODS

Experimental animal

The freshwater prawn, *M. malcolmsonii* post larvae (PL) were collected from Lower Anicut (Anakarai), Kumbakonam District, Tamil Nadu, India. They were safely transported to the laboratory in plastic bags with well aerated river water. They were acclimatized to ambient laboratory conditions for 3 weeks in large cement tank (1000 L) with ground water (pH, 7.10±0.20; total dissolved solids, 0.93±0.05 g L^{-1}; dissolved oxygen, 7.30±0.40 mg L^{-1}; BOD, 35.00±1.50 mg L^{-1}; COD, 125.0±8.50 mg L^{-1}; ammonia, 0.016±0.005 mg L^{-1}) and aerated.

Experimental feeds

Spices, such as seeds of *P. somniferum* (poppy, kasa kasa), pods of *E. cardamomum* (cardamom, elakkay), seeds of *F. vulgare* (fennel, soambu, perunjeeragam) and flower buds of *S. aromaticum* (clove, kirambu) were purchased from traditional medicinal shops at Coimbatore. These spices were individually ground to fine powders and stored at room temperature. The branded feed basal ingredients (BI), such as fish meal (25%), soybean meal (20%), groundnut oilcake (15%), wheat bran (5%) and rice bran (10%) were taken in powder forms and thoroughly mixed. Sunflower oil (2%) was used as lipid source. The selected spice powder was individually incorporated with BI in three different concentrations each at 1%, 3% and 5% by replacing the right quantity of BI. Tapioca flour (15%) and egg albumin (7%) were used as binding agents. The dough was steam cooked and cooled at room temperature. Vitamin B-complex forte with vitamin C (1%, BECOSULES® CAPSULES, Pfizer Ltd., Navi Mumbai, India) was also mixed. Sterilized water was adequately added for maintaining the mix in moist and paste form. This mix was pelletized in a manual pelletizer (Kolkata, India) fixed with 3 mm diameter mesh. The pellets were immediately dried in a thermostatic oven (M/s Modern Industrial, Mumbai, India) at 37-40ºC for one hour to quickly reduce the moisture in order to keep them intact, and then shade dried until they reach constant weight. To maintain its brittleness they were stored in airtight jars at room temperature. It is important to mention here that freshwater prawn requires 30-40% crude protein, 25-35% carbohydrate and 3-7% lipid[36]. In the present study, the proximate composition of organic matters was determined by adopting the methodology of AOAC[37]. The basal diet formulated contains 40.5% crude protein, 5.6% crude fat, 3.4% crude fibre, 9% total ash, 8.6 % moisture and 32.9% carbohydrate (total nitrogen free extract). The water stability of the feeds formulated was checked and the leaching percentage after 8 h of immersion was found to be between 26-30% by immersion and drying method.

Feeding trials

M. malcolmsonii PL (2.28±0.08 cm length and 0.11±0.04 g weight) was starved for 24 h before commencement of the feeding trial. Thirteen groups, each with 25 prawns were maintained in 25 L plastic tanks under a triplicate experimental set-up. One group served as control and fed with feed formulated using BI only, and the other groups were fed with experimental feeds prepared by incorporation of spices (*P. somniferum*, *E. cardamomum*, *F. vulgare* and *S. aromaticum*) at three different concentrations. The feed was allocated for two times per day (8:00 am and 8:00 pm) at 10% of body weight. The experiment was extended for a period of 45 days. The unfed feed, feces and moult (if any) were collected by siphoning method causing minimum disturbance to the prawns on daily basis during renewal of water. A few numbers of feeding trials were conducted then and there to meet out the requirement of experimental prawns for analysis of various parameters. For morphometric and nutritional analysis 10 prawns from each group were randomly measured and the mean was considered as a single value (mean of 10 individual measurements = one observation), and three such measurements were made to fulfill the triplicate analysis.

Nutritional indices

After the feeding trial, the growth parameters, such as survival rate (SR), length gain (LG), weight gain (WG), specific growth rate (SGR), food conversion rate (FCR) were determined by following equations of Tekinay and Davis[38]. Survival rate, SR (%) = Total No. of live PL/ Total No. of PL introduced initially × 100. Length gain, LG (cm) = Final length (cm) – Initial length (cm).

Weight gain, WG (g) = Final weight (g)–Initial weight (g). Specific growth rate, SGR (%) = $_{log}$ w2 − $_{log}$ w1 / t × 100 (where, w1 & w2 represents initial and final weight (g) respectively, and, 't' is the total number of experimental days). Food conversion rate, FCR (g) = Total quantity of feed intake (g) / Weight gain of the prawn (g).

Digestive enzymes

The whole flesh except eye stalk and exoskeleton was homogenized in ice cold distilled water and centrifuged at 10,000 rpm under 4ºC for 20 minutes. The supernatant was used as crud enzyme source. The activity of protease was estimated by the method of Furne *et al.,*[39]. One unit of enzyme activity represents the amount of enzyme required to liberate one µg of tyrosine min^{-1} under assay conditions. Amylase activity was assayed by starch-hydrolysis method of Bernfeld in which the increase in reducing power of buffered starch solutions was measured[40]. One unit of amylase activity was calculated as quantity (mg) of maltose liberated/ g of protein/ h. The activity of lipase was assayed by the method of Furne *et al.,*[39]. One unit of lipase activity was defined as the amount of free fatty acid released from triacyl glycerol per unit time was estimated by the amount of NaOH required to maintain pH constant and represented as mille equivalents of alkali consumed. For these parameters, tissues from five prawns were pooled together from each group to constitute a single observation and three such observations were made to fulfill the triplicate analysis (5 prawns/ group X 3 parameters = 15 prawns/ group X 3 replicates = 45 prawns).

Biochemical constituents

On the initial and final days, the concentrations of basic biochemical constituents, such as total protein, total carbohydrate and total lipid in the muscle of prawns were determined. Concentration of total protein was estimated by the method of Lowry *et al*., using ethanolic precipitated sample[41]. Concentration of total carbohydrate was estimated by the method of Roe using TCA extracted sample[42]. Concentration of total lipid was extracted by following the method of Folch *et al.,* and estimated by the method of Barnes and Blackstock[43,44]. For these parameters, tissues from five prawns were pooled together from each group to constitute a single observation and three such observations were made to fulfill the triplicate analysis (5 prawns/ group X 3 parameters = 15 prawns/ group X 3 replicates = 45 prawns).

Total haemocyte count

In a feeding trial at initial and final days of the experiment, 50 µl haemolymph was withdrawn in triplicate from the ventral sinus (in prawn's first abdominal segment) using a 26 gauge hypodermic needle on a 1 ml syringe. The syringe was pre-filled with 150 µl of anticoagulant (10 mM Tris-HCl, 250 mM sucrose, 100 mM sodium citrate, pH-7.6). More anticoagulant was added to make up the volume to 1 ml and the anti coagulated haemolymph was prepared. Further, a volume of 200 µl anti coagulated haemolymph was fixed with an equal volume of formalin (10%) for 30 minutes, and 100 µl of fixed haemolymph was stained with 20 µl of Rose Bengal stain (1.2% Rose Bengal in 50% ethanol) and incubated at room temperature for 20 minutes before being used to determine total haemocyte count **(**THC). THC was determined by hemocytometer (Neubauer improved, Germany) under the light microscope at RP10x (Labomed, CXR2). THC (cells $\times 10^5$ ml^{-1}) = Counted cells × depth of chamber × dilution factor / Total number of 1 mm square.

Vitamins and minerals

Concentration of ascorbic acid present in TCA extracted tissues sample was measured according to the method of Roe and Kuether[45]. Concentration of α-tocopherol present in petroleum ether-ethanol extracted tissue sample was estimated by the method of Baker *et al*.,[46]. The quantity of vitamin was expressed in µ mol/mg protein.

Contents of minerals, such as Na^+ and K^+ present in HCl digested muscle sample were estimated following the simple flame photometric method of Jeffery *et al*.,[47] using a flame photometer (Elico flame photometer, model CL 220). NaCl and KCl were used as standards. For these parameters, tissues from five prawns were pooled together from each group to constitute a single observation and three such observations were made to fulfill the triplicate analysis (5 prawns/ group X 4 parameters = 20 prawns/ group X 3 replicates = 60 prawns).

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

Enzymatic antioxidants and lipid peroxidation

The hepatopancreas of test prawns was dissected out and immediately homogenized (10% w/v) in ice-cold 50 mM Tris buffer (pH 7.4), centrifuged at 10,000 g for 20 min at 4ºC and the supernatant was used to assay the enzyme activities. Superoxide dismutase (SOD) activity was measured using pyrogallol (10 mM) autoxidation in Tris buffer (50 mM, pH 7.0) by the method of Kakkar *et al*.,[48]. The specific activity of the enzyme was expressed as units/ mg protein. Catalase (CAT) activity was measured using H_2O_2 as the substrate in phosphate buffer by the method of Sinha[49]. The activity of catalase was expressed as µ moles of hydrogen peroxide consumed/ min/ mg protein.

Lipid peroxidation (LPO) in the tissue homogenates was measured by estimating the formation of thiobarbituric acid reactive substances (TBARS) by following the method of Ohkawa *et al.*[50]. TBARS was expressed as nmoles of malondialdehyde (MDA)/ mg protein. For these parameters, tissues from five prawns were pooled together from each group to constitute a single observation and three such observations were made to fulfill the triplicate analysis (5 prawns/ group X 3 parameters = 15 prawns/ group X 3 replicates = 45 prawns).

Data between control versus experiments and between experiments were subjected to statistical analysis through one-way ANOVA and subsequent *post hoc* multiple comparison with DMRT by adopting SPSS (v11.5). The data were subjected to two-way ANOVA as well by adopting SPSS (v16.0). $P<0.05$ were considered statistically (95%) significant. All the details of statistical analyses were given in respective tables.

RESULTS

The initial length and weight of *M. malcolmsonii* was 2.28±0.08 cm and 0.11±0.04 g respectively. At the end of the feeding trail, the growth (WG and SGR) and survival were found to be higher in experimental feeds fed prawns when compared with control. In each category, the increase was maximum in *P. somniferum* (1%) incorporated feed fed prawns followed by *E. cardamomum* (1%), *F. vulgare* (3%) and *S. aromaticum* (1%). These differences were found to be statistically significant ($P < 0.05$). The growth and survival were found to be gradually decreased when concentrations of these spices were increased. Further it was confirmed by the values of FCR, just the reverse trend of WG and SGR was recorded (Table 1).

The activities of digestive enzymes, such as protease, amylase and lipase were found to be significantly increased ($P < 0.05$) in experimental feeds fed prawns groups when compared with control (Table 2). Among the three concentrations tested in each spices, *P. somniferum* (1%) incorporated feed fed prawns showed the best performance followed by *E. cardamomum* (1%), *F. vulgare* (3%) and *S. aromaticum* (1%) when compared with control.

The basic biochemical constituents, such as total protein, total carbohydrate and total lipid, and total haemocyte population (Table 3), non-enzymatic antioxidants, such as vitamin-C and E, electrolytes, such as sodium and potassium (Table 4), were found to be significantly increased ($P < 0.05$) in experimental feeds fed prawn groups when compared with control (Tables 3 and 4). Among the three concentrations tested in each spices, *P. somniferum* (1%) incorporated feed fed prawns showed the best performance followed by *E. cardamomum* (1%), *F. vulgare* (3%) and *S. aromaticum* (1%).

The activities of enzymatic antioxidants, such as SOD and CAT were not altered significantly between control and *P. somniferum*, *E. cardamomum*, *F. vulgare*, and *S. aromaticum* incorporated experimental feeds fed prawn groups (Table 5). These results reflect the fact that these spices are non-toxic at the tested concentrations. The process of LPO in *P. somniferum*, *E. cardamomum*, *F. vulgare*, and *S. aromaticum* incorporated experimental feeds fed prawns was decreased when compared with control. The decrease was gradually higher when the concentration of the spice incorporation was increased.

Maximum decrease was seen in 5% *F. vulgare* incorporation, followed by 5% *S. aromaticum*, 5% *E. cardamomum* and 5% *P. somniferum* (Table 5).

Table 1. Morphometric data and nutritional indices of *Macrobrachium malcolmsonii* PL fed with spices incorporated feeds

Spices incorporated feeds		Length (cm)	Weight (g)	WG (g)	SR (%)	SGR (%)	FCR (g)
Initial	--	2.28±0.08	0.11±0.04	--	--	--	--
Control	BI	3.30±0.18^{g}	0.30±0.07^{g}	0.19±0.02^{g}	76.00±8.00^{c}	0.99±0.59^{b}	2.94±0.16^{a}
P. somniferum	BI+1%	5.10±0.13^{a}	1.09±0.03^{a}	0.98±0.05^{a}	92.00±4.00^{a}	2.05±0.44^{a}	1.17±0.07^{f}
	BI+3%	4.40±0.11^{cd}	0.83±0.02^{bc}	0.72±0.08^{bc}	84.00±4.00^{abc}	1.99±0.34^{a}	1.41±0.12^{e}
	BI+5%	4.20±0.12^{e}	0.56±0.07^{e}	0.45±0.02^{e}	80.00±8.00^{bc}	1.38±0.65^{ab}	1.84±0.08^{bc}
E. cardamomum	BI+1%	4.80±0.12^{b}	0.88±0.06^{b}	0.77±0.07^{b}	92.00±4.00^{a}	2.05±0.30^{a}	1.42±0.11^{e}
	BI+3%	4.30±0.10^{de}	0.78±0.04^{cd}	0.67±0.03^{cd}	88.00±4.00^{ab}	1.93±0.32^{a}	1.49±0.15^{e}
	BI+5%	4.20±0.07^{e}	0.72±0.04^{d}	0.61±0.04^{d}	80.00±4.00^{bc}	1.85±0.42^{a}	1.60±0.12^{de}
F. vulgare	BI+1%	4.00±0.06^{f}	0.54±0.03^{ef}	0.43±0.03^{ef}	80.00±4.00^{bc}	1.57±0.42^{ab}	1.88±0.18^{bc}
	BI+3%	4.50±0.11^{c}	0.83±0.08^{bc}	0.72±0.09^{bc}	88.00±4.00^{ab}	1.99±0.46^{a}	1.46±0.13^{e}
	BI+5%	4.20±0.08^{e}	0.75±0.05^{cd}	0.64±0.07^{cd}	80.00±4.00^{bc}	1.89±0.43^{a}	1.54±0.09^{e}
S. aromaticum	BI+1%	4.30±0.07^{de}	0.77±0.04^{cd}	0.66±0.03^{cd}	84.00±4.00^{abc}	1.92±0.41^{a}	1.51±0.11^{e}
	BI+3%	4.20±0.08^{e}	0.62±0.06^{e}	0.51±0.02^{e}	80.00±4.00^{bc}	1.71±0.46^{ab}	1.78±0.09^{cd}
	BI+5%	3.40±0.06^{g}	0.47±0.03^{f}	0.36±0.02^{f}	80.00±8.00^{bc}	1.44±0.43^{ab}	2.05±0.14^{b}
Herbs (H)	F- value	67.09	32.16	28.78	4.34	0.51^{NS}	12.23
Concentration (C)	F- value	101.73	50.36	45.06	4.08	1.43^{NS}	16.75
C×H	F- value	35.36	33.74	30.19	2.29^{NS}	0.85^{NS}	11.59

Each value is mean ± SD (n = 3).
One-way ANOVA: Mean values within the same column sharing the different alphabetical superscripts are significantly different ($P < 0.05$); Mean values within the same column sharing the same superscript are not significantly different ($P > 0.05$); a-f, order of performance (a > b > c > d > e > f); Some of the mean sharing more than one superscripts means falls in more than one rank.
Two-way ANOVA: F-values are significant at $P < 0.05$; ^{NS}, Not statistically significant.
BI, Bsal ingredients; LG, Length gain; WG, Weight gain; SR, Survival rate; SGR, Specific growth rate; FCR, Food conversion ratio.

Table 2. Activity of digestive enzymes in *Macrobrachium malcolmsonii* PL fed with spices incorporated feeds

Spices incorporated feeds		Protease (U/mg protein)	Amylase (U/mg protein)	Lipase (U/mg protein×10^3)
Initial	--	0.26±0.03	0.15±0.04	0.73±0.07
Control	BI	0.76±0.07^{fg}	0.28±0.09^{b}	0.82±0.11^{d}
P. somniferum	BI+1%	2.85±0.11^{a}	0.65±0.09^{a}	1.82±0.21^{a}
	BI+3%	1.77±0.22^{c}	0.62±0.10^{a}	1.48±0.11^{bc}
	BI+5%	0.88±0.15^{fg}	0.51±0.20^{ab}	1.32±0.16^{bc}
E. cardamomum	BI+1%	2.40±0.21^{b}	0.64±0.16^{a}	1.61±0.14^{ab}
	BI+3%	1.62±0.17^{c}	0.61±0.19^{a}	1.44±0.17^{bc}
	BI+5%	0.98±0.11^{ef}	0.53±0.12^{a}	1.36±0.19^{bc}
F. vulgare	BI+1%	0.88±0.07^{fg}	0.41±0.04^{ab}	1.25±0.13^{c}
	BI+3%	2.17±0.17^{b}	0.63±0.10^{a}	1.50±0.16^{bc}
	BI+5%	1.25±0.22^{de}	0.57±0.13^{a}	1.39±0.19^{bc}
S. aromaticum	BI+1%	1.30±0.25^{d}	0.60±0.16^{a}	1.42±0.25^{bc}
	BI+3%	0.89±0.13^{fg}	0.53±0.14^{a}	1.34±0.13^{bc}
	BI+5%	0.66±0.09^{g}	0.40±0.06^{ab}	1.25±0.08^{c}
Herbs (H)	F- value	47.15	0.89^{NS}	2.71^{NS}
Concentration (C)	F- value	95.64	1.67^{NS}	4.16
C×H	F- value	40.06	1.25^{NS}	2.40^{NS}

Each value is mean ± SD (n = 3).
One-way ANOVA: Mean values within the same column sharing the different superscripts are significantly different ($P < 0.05$); Mean values within the same column sharing the same superscript are not significantly different ($P > 0.05$); a-f, order of performance (a > b > c > d > e > f); Some of the mean sharing more than one superscripts means falls in more than one rank.

Two-way ANOVA: F-values are significant at $P < 0.05$; ^{NS}, Not statistically significant.
BI, Bsal ingredients.

Table 3. Concentrations of basic biochemical constituents and THC in *Macrobrachium malcolmsonii* PL fed with spices incorporated feeds

Spices incorporated feeds		Total Protein (mg/g wet wt.)	Total Carbohydrate (mg/g wet wt.)	Total Lipid (mg/g wet wt.)	THC (Cells×10^5 ml^{-1})
Initial	--	75.73±3.05	19.05±1.25	4.56±0.32	45.67±2.14
Control	BI	124.25±3.81^{h}	28.03±1.67^{ef}	9.42±1.05^{f}	55.75±3.10^{g}
P. somniferum	BI+1%	233.16±4.19^{a}	46.44±2.81^{a}	27.25±1.13^{a}	79.46±3.65^{a}
	BI+3%	203.50±3.94^{b}	38.34±2.16^{bc}	22.49±1.04^{bc}	77.88±3.51^{bc}
	BI+5%	163.27±2.45^{fg}	25.31±2.59^{f}	14.91±1.75^{e}	64.67±2.50^{ef}
E. cardamomum	BI+1%	205.18±4.97^{b}	40.07±1.84^{b}	24.26±1.34^{b}	75.39±4.38^{abc}
	BI+3%	196.85±2.81^{c}	35.66±1.95^{cd}	20.12±1.56^{cd}	73.45±3.75^{abcd}
	BI+5%	168.87±3.56^{f}	33.62±1.04^{d}	15.38±1.87^{e}	71.23±4.60^{bcde}
F. vulgare	BI+1%	161.74±3.26^{g}	25.10±1.18^{f}	14.70±1.92^{e}	64.67±2.50^{ef}
	BI+3%	205.03±4.71^{b}	39.22±1.87^{b}	22.73±1.35^{bc}	69.54±3.78^{cde}
	BI+5%	179.34±3.14^{e}	35.08±1.28^{cd}	16.21±1.31^{e}	65.76±3.12^{ef}
S. aromaticum	BI+1%	186.29±4.08^{d}	35.26±1.72^{cd}	19.24±1.92^{d}	67.42±3.69^{de}
	BI+3%	164.27±3.23^{fg}	30.14±1.04^{e}	14.95±1.58^{e}	64.76±4.18^{ef}
	BI+5%	128.86±2.73^{h}	21.05±1.36^{g}	13.74±1.67^{e}	60.38±4.35^{fg}
Herbs (H)	F- value	196.17	36.65	21.63	20.10
Concentration (C)	F- value	355.83	68.59	54.39	15.57
C×H	F- value	97.21	45.93	16.64	3.38

Each value is mean ± SD ($n = 3$).
One-way ANOVA: Mean values within the same column sharing the different superscripts are significantly different ($P < 0.05$); Mean values within the same column sharing the same superscript are not significantly different ($P > 0.05$); a-f, order of performance (a > b > c > d > e > f); Some of the mean sharing more than one superscripts means falls in more than one rank.
Two-way ANOVA: F-values are significant at $P < 0.05$.
BI, Basal ingredients; THC, Total hemocyte count.

Table 4. Concentrations of vitamins and minerals in *Macrobrachium malcolmsonii* PL fed with spices incorporated feeds

Spices incorporated feeds		Vitamin - C (µ mol/mg protein)	Vitamin - E (µ mol/mg protein)	Sodium (mg/g)	Potassium (mg/g)
Initial	--	17.28±1.78	8.27±1.02	0.053±0.002	0.093±0.006
Control	BI	43.66±2.54^{c}	20.98±2.12^{f}	0.306±0.040^{h}	0.440±0.025^{cd}
P. somniferum	BI+1%	71.94±4.80^{a}	50.70±3.23^{a}	0.830±0.091^{a}	0.597±0.094^{a}
	BI+3%	67.00±4.53^{ab}	44.76±3.90^{bc}	0.748±0.063^{abdc}	0.554±0.023^{abc}
	BI+5%	60.92±3.11^{b}	40.31±3.92^{dce}	0.581±0.094^{efg}	0.452±0.011^{bcd}
E. cardamomum	BI+1%	70.54±3.94^{a}	47.78±3.52^{ab}	0.812±0.095^{ab}	0.574±0.054^{ab}
	BI+3%	67.53±3.50^{ab}	43.82±3.01^{bc}	0.710±0.055^{abcde}	0.539±0.067^{abcd}
	BI+5%	61.68±4.22^{b}	42.40±3.13^{bc}	0.623±0.074^{defg}	0.497±0.035^{abcd}
F. vulgare	BI+1%	60.82±4.95^{b}	37.00±2.23^{de}	0.548±0.053^{fg}	0.551±0.098^{abc}
	BI+3%	69.51±3.17^{a}	45.85±2.00^{abc}	0.763±0.032^{abc}	0.568±0.084^{ab}
	BI+5%	66.24±3.83^{ab}	42.03±2.99^{cd}	0.658±0.070^{cdef}	0.512±0.047^{abcd}
S. aromaticum	BI+1%	67.19±4.16^{ab}	42.56±2.65^{bc}	0.684±0.052^{bcde}	0.525±0.097^{abcd}
	BI+3%	61.03±3.21^{b}	41.18±2.50^{cde}	0.613±0.061^{efg}	0.466±0.082^{bcd}
	BI+5%	60.15±3.55^{b}	36.58±1.21^{e}	0.514±0.090^{g}	0.424±0.043^{d}
Herbs (H)	F- value	1.85^{NS}	6.21	5.21	2.20^{NS}
Concentration (C)	F- value	5.97	7.00	11.13	5.56
C×H	F- value	3.40	5.03	4.80	0.42^{NS}

Each value is mean ± SD ($n = 3$).
One-way ANOVA: Mean values within the same column sharing the different superscripts are significantly different ($P < 0.05$); Mean values within the same column sharing the same superscript are not significantly different ($P > 0.05$); a-f, order of performance (a > b > c > d > e > f); Some of the mean sharing more than one superscripts means falls in more than one rank.

Two-way ANOVA: F-values are significant at $P < 0.05$; ^{NS}, Not statistically significant.
BI, Basal ingredients.

Table 5. Activities of enzymatic antioxidants (SOD and CAT) and lipid peroxidation (LPO) in *Macrobrachium malcolmsonii* PL fed with spices incorporated feeds

Spices incorporated feeds		SOD (µ mol H_2O_2 consum/ min/ mg protein)	CAT (U/ mg protein)	LPO (n mol MDA/ mg protein)
Initial	--	20.47±1.74	13.78±1.58	0.78±0.13
Control	BI	58.32±2.09^{a}	27.49±1.25^{a}	2.77±0.15^{a}
P. somniferum	BI+1%	59.79±2.37^{a}	29.87±1.14^{a}	2.72±0.18^{ab}
	BI+3%	58.74±2.93^{a}	28.42±1.65^{a}	2.48±0.22^{abcd}
	BI+5%	57.46±2.72^{a}	26.86±1.79^{a}	2.27±0.26^{cdef}
E. cardamomum	BI+1%	59.73±3.58^{a}	29.83±2.73^{a}	2.60±0.14^{abc}
	BI+3%	58.06±2.80^{a}	28.32±2.88^{a}	2.43±0.21^{abcde}
	BI+5%	58.22±3.02^{a}	27.36±2.86^{a}	2.15±0.15^{def}
F. vulgare	BI+1%	57.42±3.72^{a}	26.80±1.93^{a}	2.40±0.17^{abcde}
	BI+3%	59.71±2.15^{a}	28.75±2.92^{a}	2.06±0.23^{ef}
	BI+5%	58.37±2.44^{a}	27.41±2.07^{a}	1.98±0.22^{f}
S. aromaticum	BI+1%	59.71±3.11^{a}	28.69±2.91^{a}	2.42±0.14^{abcde}
	BI+3%	58.39±3.78^{a}	27.45±2.16^{a}	2.13±0.18^{def}
	BI+5%	58.73±3.56^{a}	27.83±2.38^{a}	2.06±0.21^{ef}
Herbs (H)	F- value	0.03^{NS}	0.24^{NS}	6.04
Concentration (C)	F- value	0.28^{NS}	1.12^{NS}	14.03
C×H	F- value	0.32^{NS}	0.56^{NS}	0.22^{NS}

Each value is mean ± SD ($n = 3$).
One-way ANOVA: Mean values within the same column sharing the different superscripts are significantly different ($P < 0.05$); Mean values within the same column sharing the same superscript are not significantly different ($P > 0.05$); a-f, order of performance (a > b > c > d > e > f); Some of the mean sharing more than one superscripts means falls in more than one rank.
Two-way ANOVA: F-values are significant at $P < 0.05$; ^{NS}, Not statistically significant.
BI, Basal ingredients; SOD, Superoxide dismutase; CAT, Catalase; LPO, Lipid peroxidation;
MDA, Melondialdehyde.

DISCUSSION

In this study, herbal incorporation resulted in effective feed utilization due to enhanced activity of digestive enzymes, which leads to better survival and growth with enhanced tissue biochemical constituents in *M. malcolmsonii*. Therefore, the formulated feeds were digestible by *M. malcolmsonii*. According to Mukhopadhyay *et al*., dietary protein utilization is mainly controlled by its amino acid composition, calorie content, digestibility, physiological state and size of the individual[51]. It has been reported that certain herbs, such as *Massa medicata, Cratae gifructus, Artemisia capillaries, Cnidium officinale* and *W. somnifera* promoted feed utilization and promoted general health for good growth in aquatic organisms[52-54]. In this study, increased vitamin-C and E clearly indicate the fact that the general health of *M. malcolmsonii* was improved due to incorporation of spices as these vitamins are potent antioxidants. The potency of vitamin-C and E in scavenging reactive oxygen species (ROS) or oxygen free radicals, such as hydroxyl, perhydroxyl, peroxyl and nitric oxide have been reported[55-58]. In this study, the increased Na and K levels suggest that they were also aided for better survival and growth of *M. malcolmsonii* as they are the main regulators of osmotic pressure in organisms. It is important to mention here that minerals like Ca, Cu, Mg, P, Na, K, Cl, Se and Zn are essential for growth in addition to maintenance of acid-base balance, membrane potential and body metabolism[59-62].

In the present study, *P. somniferum*, *E. cardamomum*, *F. vulgare*, and *S. aromaticum* were acted as immune stimulants and increased the haemocyte population, which indicates prevalence of good host defense mechanisms. The haemocyte population is used as an indicator of crustacean health status[63]. It has been reported that incorporation of anthraquinone taken from *Rheum officinale* improved activities of haemolymph lysozyme, alkaline phosphatase and antioxidation abilities, which leads to improved growth In *M. rosenbergii*[64].

It has also been reported that herbs, like *O. sanctum*, *W. somnifera* and *M. fragrans* improved phagocytic activity, serum bactericidal activity, albumin-globulin ratio and leukocrit against *Vibrio harveyi* in the grouper, *Epinephelus tauvina*[65]. Similarly, extracts of *Viscum album*, *Urtica dioica* and *Z. officinalis* have improved the non-specific defense mechanisms, including extracellular and intra cellular respiratory burst activities, phagocytosis in blood leukocytes, total plasma protein, specific growth rate and condition factor in *Epinephelus tauvina*[66].

In this study, increased activities of digestive enzymes in *M. malcolmsonii* due to incorporation of spices, suggests enhanced food consumption and absorption of nutrients and therefore, elevation of basic biochemical constituents, such as total protein, vitamins and minerals were resulted. Further, the prevailed less predominant lipid peroxidation in spice incorporated feeds fed prawn suggests limited production of ROS. Therefore, insignificant changes in activities of SOD and CAT were recorded. Thus, these states forced us to conclude that these spices were not induced any oxidative stress in *M. malcolmsonii* at test concentrations. Furthermore, the elevated haemocyte population ensures prevalence of good host defence, which favours good health and ultimately resulted in better survival and growth of *M. malcolmsonii*. Similar increases in growth performance, activities of digestive enzymes, and concentrations of biochemical constituents have also previously been reported in *M. rosenbergii* fed with medicinal herbs[10-16]. Since *P. somniferum*, *E. cardamomum*, *F. vulgare*, and *S. aromaticum* produced appreciable performance in *M. malcolmsonii*, they can be incorporated in aqua feed formulations for sustainable development of freshwater prawn culture.

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