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Allium sativum-, *Zingiber officinale*- and *Curcuma longa*-Induced Digestive and Antioxidant Enzyme Activities in *Macrobrachium rosenbergii* Post Larvae

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ABSTRACT

The digestive and antioxidant effects of three Indian medicinal herbs *Allium sativum* (AS), *Zingiber officinale* (ZO) and *Curcuma longa* (CL) were studied at enzyme level in *Macrobrachium rosenbergii* post-larvae (PL) to assess the growth promoting and survival enhancement properties of these herbs. The PL were given 10 different diets containing AS, ZO and CL each at 1, 3 and 5% levels for 90 days under laboratory conditions. Experiments were conducted in parallel with a control diet having no herb supplementation. At the end of the trial, significant increase in weight gain (WG) ($P < 0.046$) was found in the groups fed with 5% ZO. The survival rate increased significantly with 5% CL supplementation ($P < 0.049$). ZO (5%) significantly increased the activity level of protease ($P < 0.048$), amylase ($P < 0.204$) and lipase ($P < 0.003$). CL (5%) supplementation significantly increased the activity level of two antioxidant enzymes, namely, superoxide dismutase (SOD) ($P < 0.045$) and catalase (CAT) ($P < 0.004$). AS (5%) supplementation significantly increased the activity of the antioxidant enzyme glutathione peroxidase (GPX) ($P < 0.072$). Therefore, it is concluded that ZO can promote growth by improving the digestive enzyme activities (protease, amylase and lipase) and CL is able to enhance the survivability by improving the antioxidant enzyme (SOD and CAT) activities.

Keywords: *Allium sativum*, Antioxidant enzymes, *Curcuma longa,* Digestive enzymes and *Zingiber officinale*

1. INTRODUCTION

In the aquaculture industry, animal growth and survival rates play a vital role. Feed formula is a major input in the hatchery and the availability of cost-effective feeds play an important role in the aquaculture industry (Chunchom *et al.,* 2010). Some of the natural products find their use not as pharmaceuticals but as a novel class of dietary supplements or nutraceuticals that fall well into the concept of functional food (Ajith, 2010). Digestive enzymes can be used as an indicator of digestion and nutritional status at an early life stage of many aquatic species (Hamza *et al.,* 2007; Manush *et al.,* 2013). Of the many digestive enzymes, the activities of protease, amylase and lipase have been frequently studied to evaluate the nutritional status and growth performance of various fish larvae and juveniles (Shan *et al.,* 2009). Growth is obtained by the oxidation of the three main classes of foodstuffs, namely, carbohydrates, fats and proteins. Available nutrients are metabolised using oxygen for energy production. Accordingly, ROS (reactive oxygen species) are continually produced as toxic by-products of normal metabolism from various endogenous processes. They can attack biomolecules instantly and indiscriminately, leading to oxidative stress. Antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) are important antioxidants that limit the oxidative assault to various biomolecules (Verma and Vinayak, 2008; Manju *et al.,* 2009). Oral supplementations of medicinal plants have been used for improving growth and survival rates (Citarasu,

2010) to avoid any ill effects of synthetic compounds (Rattanachaikunsopon and Phumkhachorn, 2009). On account of this, three medicinal plants *Zingiber officinale*, *Allium sativum* and *Curcuma longa* were selected for oral supplementary use in *Macrobrachium rosenbergii* post-larvae (PL) culture.

2. MATERIALS AND METHODS

The PL-15 of the freshwater prawn *M. rosenbergii* were purchased from the Rosen Fisheries, at the Thrissur district of Kerala in South India. Before trial, the prawns were acclimatised to experimental condition for 2 weeks, during which they were fed *ad libitum* with boiled egg albumin, phytoplankton, cultured Artemia and commercial feed (scampi) thrice a day alternatively at 10% body weight. Water was routinely changed every day apart from providing artificial aeration. PL of 0.07–0.1 g weight and 1.5–2.1 cm length were chosen as the experimental animals for the study. The prepared diets contained two major ingredients - the basal and the supplementary ingredients. All of the basal feed ingredients (fish meal, groundnut oil cake, coconut oil cake, wheat bran, rice bran, maize bran and tapioca flour) and supplementary feed ingredients (Fresh *Allium sativum* (garlic) bulbs and rhizomes of *Zingiber officinale* (ginger) and *Curcuma longa* (turmeric) were purchased from the local merchants of Coimbatore district, Tamil Nadu, India. Bulbs of garlic and rhizomes of ginger and turmeric were de-skinned and chopped finely for quick evaporation of moisture and to minimise degradation of the material. They were then allowed to shade dry to prevent excessive loss of micronutrients. All the ingredients were individually grounded to a fine powder and then mixed according to the formulation equated to 40% protein of the diets. Diet without herbal supplementation served as control. Feeds were prepared using hand pelleting method. The body weight of all the animals was recorded at the beginning of the experiment. One trial consisted of 10 individual groups in triplicates of the PL of the freshwater prawn *M. rosenbergii* (10 × 3) with 25 PL in each group (10 × 3 × 25). The prawns were given test diet for 2 days to acclimatise them, after which they were assigned to three treatments (1, 3 and 5%) of herb-supplemented diets. Group 1 served as control, which was fed with basal diet having no supplementation. The animals were then given the particulate feeds every day at a daily rate of 1% of their live weights. Everyday unfed feed and faeces were separately collected and quantified. The sampling of the animals was done after 90 days of feeding trials. Weight gain and survival rate were calculated (Cerezo-Valverde *et al.*, 2008). The digestive tract and muscle tissues were taken for biochemical analysis. At the end of the feeding trial, growth and survival performances, indices of feed quality and indices of protein 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 × 100

Weight gained (WG) = Final weight – Initial weight

Feed intake (FI) = Amount of feed consumed

For analysis of digestive enzymes, the whole digestive tract was isolated and homogenised at 4°C using ice-cold distilled water in the ratio 1:5 (W/V). The contents were centrifuged at 4°C and 10000 rpm for 20 min and the supernatant was used for the estimation of digestive enzymes. For analysis of antioxidant enzymes, muscle tissues were homogenised (10% W/V) in ice-cold 50 mM Tris buffer (pH 7.4), centrifuged at 10000 rpm for 20 min at 4°C and the supernatant was used to assay the enzyme activities. Total protein content of the supernatant was determined using the method given by Lowry *et al.* (1951). The activity of protease was assayed in 0.2 mg glycine-HCL (pH-2.0) buffer with 1% casein as substrate (Sarath *et al.*, 1989). Amylase activity was assayed in 0.2 M citrate/phosphate buffer solution (pH 7.0) with starch (50g) as substrate (Bernfeld, 1955). Lipase activity was determined through hydrolysis of phenyl myristate, adapted from Albro *et al.* (1985). The SOD activity was measured using pyrogallol (10 mM) autoxidation in Tris buffer (50 mM, pH 7.0) following the method of Marklund and Marklund (1974). The CAT activity was measured using H_2O_2

as the substrate in phosphate buffer, following the method of Sinha (1972). The GPX activity was assayed following the method of Rotruck *et al.* (1973). All data were represented as mean ± standard deviation. Significant differences between the mean values were statistically analysed using Student's *t*-test (Zar, 1984) to compare the mean values of different parameters in controls with those of each experimental groups using SPSS software (version, 11.5) of the IBM Company, USA.

3. RESULTS AND DISCUSSION

A $P < 0.046$ level of increase in WG was observed with 5% ZO supplementation. A $P < 0.049$ level of increase in SR was seen with 5% CL supplementation. $P < 0.048$ level of increase in protease activity, $P < 0.204$ level of increase in amylase activity and $P < 0.003$ level of increase in lipase activity were observed with 5% ZO supplementation. $P < 0.045$ level of increase in SOD activity, $P < 0.004$ level of increase in CAT activity was seen with 5% CL supplementation. $P < 0.072$ level of increase in GPX activity was observed with 5% AS supplementation. Improved protease, amylase and lipase activities in the groups fed with 5% ZO reflects the functional effects of the bioactive compounds (Gingerol, shogaols, zingerone/vanillylacetone, paradol and curcuminoids) present in it which are responsible for the stomachic, laxative, gastric emptying enhancer, appetiser, antiemetic, antidyspepsic, antidiarrhoeal and anticolic properties (Ghayur and Gilani, 2005), including the antioxidant (Kim *et al.*, 2007) and the antimicrobial (Ajith *et al.*, 2008) properties. The digestive enzyme activity increases with increased ZO supplementation (Table 2). Similar results were obtained with Venketramalingam *et al.* (2007) and Sambhu and Jayaprakas (2001). AS-induced amylase and lipase activities have been reported by Ghosh *et al.* (2010). Increased CL supplementation does not correlate with increased digestive enzyme activity. In such cases, feed intake can be affected by palatability (Sudaryono, 2006). Growth is affected by nutrients in the diet (Verri *et al.*, 2001), practically accomplished with feed intake (Bhavan *et al.*, 2010). A decline in feed intake results in unavailability of the nutrients in the diet for intake or for digestive enzyme activity. Digestive enzyme activity could improve feed intake (Venketramalingam *et al.*, 2007), resulting in increased growth rate by increasing weight (Supannapong *et al.*, 2008). Increased digestive activity correlates with increased feed intake (1.78±0.10) compared with the control (1.38±0.10) and weight gain (1.37±0.45) compared with control (0.25±0.04; Table 1). Digestive absorption of ZO is explained from the reduced faecal output (16.82±1.94) against the control (39.53±2.38; Table 1).

Grown cells are equipped with different kinds of mechanisms to fight against ROS and to maintain the redox homeostasis of cell. The antioxidant enzymes SOD, CAT and GPX play important roles in scavenging the free radicals and preventing cell injury (Chanda and Dave, 2009). GPX together with SOD and catalase protect cells against damage caused by free radicals

Table 1: Weight gain and survival rate of the control and experimental groups after 90 days

Parameters	Group-1 (Control)	Group-2 (1% AS)	Group-3 (3% AS)	Group-4 (5% AS)	Group-5 (1% CL)	Group-6 (3% CL)	Group-7 (5% CL)	Group-8 (1% ZO)	Group-9 (3% ZO)	Group-10 (5% ZO)
Initial weight (g)	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
Final weight (g)	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
Weight gain (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
Survival rate (%)	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 intake (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
Faecal 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

Each value is mean± SD of triplicate observations.
Significance (*P*<) of paired samples *t*-test
AS-*Allium sativum*, CL-*Curcuma longa*, ZO-*Zingiber officinale*, SD-standard deviation

Table 2: Activities of digestive enzymes of *M. rosenbergii* fed with different herb-incorporated feeds for a period of 90 days

Parameters	Group-1 (Control)	Groups: Experimental: Group-2 (1% AS)	Group-3 (3% AS)	Group-4 (5% AS)	Group-5 (1% CL)	Group-6 (3% CL)	Group-7 (5% CL)	Group-8 (1% ZO)	Group-9 (3% ZO)	Group-10 (5% ZO)
Protease (U/mg protein)	1.00±0.05	1.54±0.44	1.76±0.09	1.92±0.06	1.36±0.1	1.40±0.25	1.68±0.26	1.70±0.08	1.87±0.04	2.00±0.06
Amylase (U/mg protein)	0.81±0.03	1.06±0.078	1.16±0.09	1.25±0.06	1.05±0.11	1.10±0.01	1.21±0.13	1.19±0.01	1.27±0.07	2.00±0.25
Lipase (U/mg protein)	0.62±0.09	0.67±0.06	0.87±0.12	1.56±0.59	1.04±0.15	1.66±0.18	1.68±0.09	1.44±0.11	1.67±0.38	1.70±0.11
Total protein in homogenate (mg)	59.74±6.69	68.11±6.54	88.57±9.46	97.47±7.84	70.29±2.95	60.24±4.53	57.51±1.95	85.86±5.70	90.71±2.62	104.58±9.88

Each value is mean±SD of triplicate observations.
Significance (*P*<) of paired samples *t*-test
AS-*Allium sativum*, CL-*Curcuma longa*, ZO-*Zingiber officinale*, SD-standard deviation

Table 3: Activities of antioxidant enzymes of *M. rosenbergii* fed with different herb-incorporated feeds for a period of 90 days

Parameters	Group-1 (Control)	Groups: Experimental: Group-2 (1% AS)	Group-3 (3% AS)	Group-4 (5% AS)	Group-5 (1% CL)	Group-6 (3% CL)	Group-7 (5% CL)	Group-8 (1% ZO)	Group-9 (3% ZO)	Group-10 (5% ZO)
SOD (µmol H_2O_2 consumed/min/mg protein)	7.71±0.25	8.11±0.54	9.17±1.05	11.59±1.63	9.73±0.47	10.68±0.81	13.3±0.11	9.50±0.51	10.61±0.17	11.64±0.65
CAT (U/mg protein)	8.42±1.12	11.12±1.11	12.25±0.66	13.20±1.27	12.45±0.61	16.35±1.00	24.87±1.26	11.22±0.74	12.57±0.85	15.17±0.73
GPX (µg of GSH utilised/min/mg protein)	1.55±0.01	3.36±1.03	4.97±0.67	7.10±0.63	2.65±0.72	3.40±0.62	4.70±0.41	2.08±0.56	2.22±0.63	4.55±0.49

Each value is mean±SD of triplicate observations.
Significance (*P*<) of paired samples *t*-test.
AS-*Allium sativum*, CL-*Curcuma longa*, ZO-*Zingiber officinale*, SOD-superoxide dismutase, CAT-catalase, GPX-glutathione peroxidase, GSH-Reduced glutathione, SD-standard deviation

and hydroperoxides or lipoperoxides (Saxena and Jaiswal, 2007). The activities of antioxidant enzymes are presented in Table 3. A reduction in the survival rate in the groups fed with the control diet (62.00±2.83) is a consequence of reduction in the enzymatic antioxidants against the groups fed with CL (83.66±0.47; Table 1) in which the SOD (13.3±0.11) and CAT (24.87±1.26) activities were found to be higher compared with the other groups (Table 3). C-glutamyl-S-allyl-L-cysteine, allicin, diallyl sulphide, diallyl disulphide, diallyl trisulphide and ajoene are the bioactive compounds present in AS that are responsible for the antioxidant properties (El-Demerdash *et al.,* 2005). Curcumin is an active principle compound present in CL responsible for the antioxidant properties (Menon and Sudheer, 2007). Curcumin is the principle compound of CL (Hatcher *et al.,* 2008). In *in vitro* conditions, curcumin can significantly inhibit the generation of ROS like superoxide anions, H_2O_2 and nitrite radical generation by activated macrophages, which play an important role in inflammation. The presence of selenium in AS (Obioha and Suru, 2009) explains the improved activity of GPX with AS supplementation. Selenium plays an important role in GPX activity (Liu *et al.,* 2007). Reduced feed intake could probably reduce the rate of metabolism (Hwang and Lin, 2002). Consequently, the production of ROS is reduced and thus the survival rate is reflected to improve at a lower state of oxidative stress with 5% CL supplementation. Primarily, the influence of medicinal herbs forms a major basis for improved survival rate.

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