World Journal of Fish and Marine Sciences 4 (3): 301-307, 2012
ISSN 2078-4589

DOI: 10.5829/idosi.wjfms.2012.04.03.62120

Effects of *Zingiber officinalis* and *Cyanodon dactylon* on the Growth Performance and Immune Parameters of *Macrobrachium rosenbergii*

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Abstract: To investigate the effects of *Zingiber officinalis* and *Cyanodon dactylon* supplementation on the growth performance and immune parameters of *Macrobrachium rosenbergii* juveniles, five experimental diets, including a control basal diet and four treated diets with 1.5 and 3% *Z. officinalis* ; 2 and 4% *C. dactylon* were prepared. Prawn fed with control and supplemented diet for five weeks. Growth and immune parameters were evaluated. Juveniles fed with *Z. officinalis* and *C. dactylon* showed significant increase in SGR, CF, WGR, LGR and FCR compared with those of control along the whole experiment. Prawn fed all doses of *Z. officinalis* and *C. dactylon* revealed significantly higher THC, HV, DHC, PR and PI as compared with control at 4 and 5 weeks. Histopathologically, hepatopancreas and stomach of juveniles fed with *Z. officinalis* and *C. dactylon* showed normal histological structure. The results indicated that *Z. officinalis* and *C. dactylon* inclusion with a basal diet had the potential to improve the growth and immune response of prawns.

Key words: Herbals % Growth % Immunostimulant % Prawn

INTRODUCTION

The giant freshwater prawn, *Macrobrachium rosenbergii* is the most popular prawn species used for commercial farming and has been transported to many parts of the world including South America and China [1]. At the global level, people have understood the bad effect of chemical products and they are now shifting to natural products [2]. Natural plant products have been reported to promote various activities like anti-stress, growth promotion, appetite stimulation, tonic and immunostimulation and to have antimicrobial properties in finfish and shrimp [3].

Zingiber officinalis is beneficial to growth and immune systems in aquatic animals. Ginger rhizomes contain a number of active ingredients as ginger oil, gingerols, which can be converted to shogaols, zingerone and paradol [4]. *Cyanodon dactylon* has been used to control various diseases like diabetes, ulcer, cardiac arrhythmia and diarrhea [5,6]. *C. dactylon* whole plant contains crude proteins, carbohydrates, mineral constituents, $-sitosterol, flavanoids and phytosterols [7].

The circulating haemocytes play extremely important roles not only by direct sequestration and killing of infectious agents but also by synthesis of bioactive molecules and there appears to be participation between all cell types [8]. Previous research indicated that the total haemocyte count (THC), differential haemocyte count (DHC) are commonly used as functional parameters for evaluating the immune potential [9]. Crustacean hemocytes, including hyaline, semi-granular and granular cells, are involved in phagocytosis and the production of melanin via the prophenoloxidase (proPO) system [10]. To investigate the effects of *Zingiber officinalis* and *Cyanodon dactylon* supplementation on the growth performance and immune parameters of *Macrobrachium rosenbergii* juveniles.

MATERIALS AND METHODS

Freshwater Prawns: A total of about 300 *M. rosenbergii* Juveniles with an average body weight 0.3g were obtained from Maryout Project and Company for fish farms, Alexandria, Egypt. The prawn stocked in cement ponds supplied with brackish water of 12 ppt salinity that was

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decreased gradually until reached 0.5-1 ppt salinity within one month. Green house ponds were prepared and water parameters were adjusted at 30°C, pH 7and 0.5-1 ppt salinity. Juveniles were fed with basal diet (crude protein 30%) (Maryout Company) at rate of 3% body weight [11]. Excess feed and excreta were siphoned daily along the acclimation period. Later on, Juveniles were divided and stocked in cement ponds at a rate of 40 juveniles/pond and left for 3 days for acclimation.

Experimental Design

Preparation of Experimental Diets: Rhizomes of *Z. officinalis* [12] *and* whole plant of *C. dactylon*[13] were shade-dried and ground into fine powder at Agricultural Botany Department, Faculty of Agriculture, Benha University. Five experimental diets were prepared. Fine powdered basal diet was divided into five portions first two portions incorporated with 1.5 and 3% (w/w) *Z. officinalis* respectively, the second two portions mixed with 2 and 4% (w/w) *C. dactylon* respectively and the last portion kept free without any additives (control). Suitable amount of water was added to form moist dough then pelleted, allowed to dry at room temperature, then packed in clean dry plastic containers and kept tightly closed at 4°C until use.

Effect of Z. Officinalis and C. Dactylon on the Growth and Immune Parameters: Five groups each of 40 juveniles in two replicates were used, a control group was fed with basal diet and the four treated groups fed with diet supplied with *Z. officinalis* 1.5, *Z. officinalis* 3%, *C. dactylon* 2% and *C. dactylon* 4% for 5 weeks.

Determination of Growth Parameters: Sample of five juveniles were taken from each group at the end of 2, 3, 4 and 5 weeks after the start of feeding for evaluation of growth performance parameters.

Weight gain rate (WGR), Length gain rate (LGR), Feed conversion ratio (FCR) were calculated according to Bo Liu *et al.*[14]. Moreover, Specific growth rate (SGRs) and condition factor (K) were determined as described by Laird and Needham [15].

Measurement of Immune Related Parameters: Hemolymph was collected and divided in two portions one was mixed with Alsever's solution according to FAO [16] with slight modification {Citric acid (0.055 g), Sodium citrate (0.8 g), Glucose (2.05 g), Nacl (0.64 g), EDTA (4 g) and 100 ml Distilled water, pH (7)} for estimation of Total Hemocytic Count (THC), Hemocyte Viability (HV) and Phagocytic activity, while the other portion was mixed with 10% formalin for determination of the Differential Hemocytic Count(DHC). Immune related parameters such as THC, HV, DHC and Hemocytes phagocytosis were measured in all groups at 4 and 5 weeks.

- THC and HV were measured following the method of Cheng *et al.*[17]:

THC = Number of counted haemocytes in the 4 corners ×2×¼×10^4×the dilution factor.

HV (%) = (Total number of viable cells /total number of cells) × 100

- DHC was measured according to Abdel hameed [18].

Phagocytic Index (PI) was measured according to Itami *et al.* [19] with minor modification as following: PI = 100 × (number of haemocytes engulfed beads / total number of haemocytes) × (number of beads that were engulfed by the haemocytes / total number of haemocytes).

- Phagocytic rate (PR) was calculated according to Cheng *et al.*[17]

PR = (Phagocytic Hemocytes / Total Hemocytes) × 100.

Sampling and Histopathological Examination: Soon after scarification, specimens were taken at 3, 4 and 5 weeks from start of feeding from all groups, fixed in Davidson's Fixative and then sectioned and stained according to Banchroft *et al.* [20].

Statistical Analysis: Results were analyzed using SPSS (version 16.0) software. One way ANOVA and Duncan's multiple range tests were used to determine the significance of differences between groups. All the results were expressed as means ± standard error (M ± SE) and significant differences were expressed at a significance level of $P<0.05$.

RESULTS

Effect of Z. Officinalis and C. Dactylon on the Growth Parameters: Growth performance parameters including SGR, CF, WGR, LGR and FCR of juveniles from all groups were significantly increased compared to the control along the whole experiment period (Fig, 1). In addition the survival rate of *M. rosenbergii* among all groups in each period of time was similar (100%).

Table 1: Effect of *Zingiber officinalis* and *Cyanodon dactylon* incorporated diets on Total Hemocyte Count (10^6/ml) and Hemocyte Viability (%) of *M. rosenbergii* at 4 and 5 weeks.

	Total Hemocyte Count		Hemocyte Viability	
Treatments	4 (w_s)	5 (w_s)	4 (w_s)	5 (w_s)
Control	7.8±0.057^e	5.7±0.057^e	48.8±0.057^d	65.21±0.005^e
Z. officinalis 1.5%	20.1±0.057^b	22.2±0.057^c	100.0±0.577^a	82.64±0.005^c
Z. officinalis 3%	12.9±0.057^a	15.6±0.057^a	59.7±0.577^a	74.78±0.005^b
C. dactylon 2%	12.6±0.057^c	9.9±0.057^b	54.6±0.057^b	84.15±0.005^d
C. dactylon 4%	16.8±0.057^d	15.3±0.057^d	100.0±0.057^c	82.50±0.005^a

Mean values (± SE) at the same exposure time with different superscript letters are significantly different at level of $P< 0.05$.

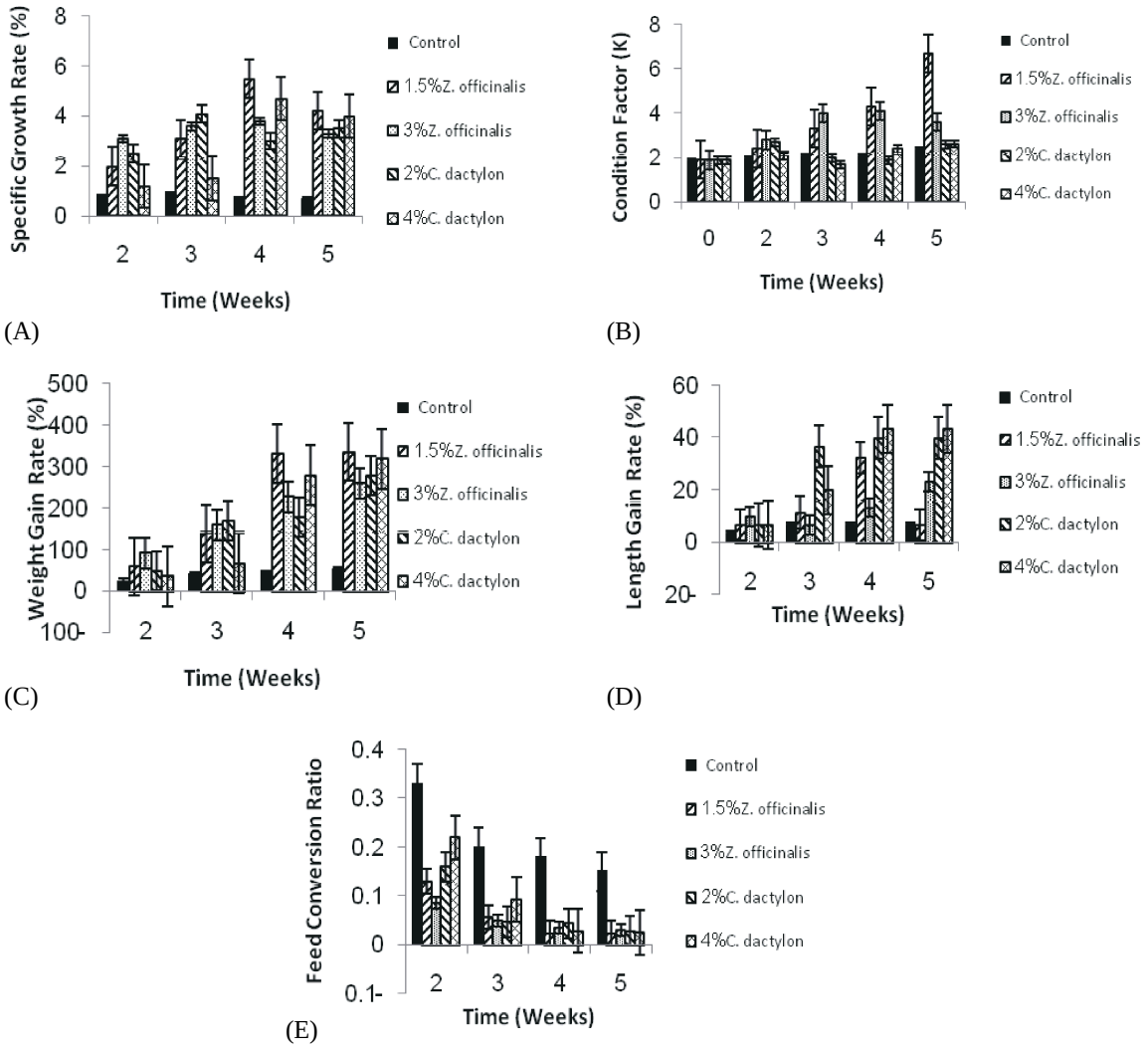
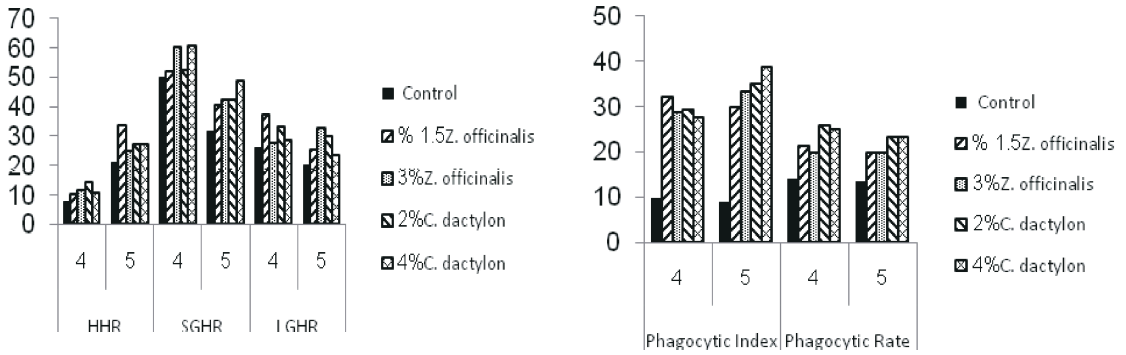


Fig. 1: Effect of Zingiber officinalis and Cyanodon dactylon incorporated diets on Specific growth rate (A), Condition factor (B), Weight gain rate (C), Length gain rate (D) and Feed conversion ratio (E) of M. rosenbergii for period of 5 weeks.

Effect of Z. Officinalis and C. Dactylon on the Immune Parameters: THC and HV of juveniles from all groups were significantly increased compared to the control at 4 and 5 weeks (Table, 1). Moreover, DHC all groups were significantly increased compared to the control at 4 and 5 weeks. It was clear that small granular hemocytes were the



(A) (B)

Fig. 2: Effect of Zingiber officinalis and Cyanodon dactylon incorporated diets on Differential Hemocytic Count and phagocytic activity of M. rosenbergii at week 4 and 5.

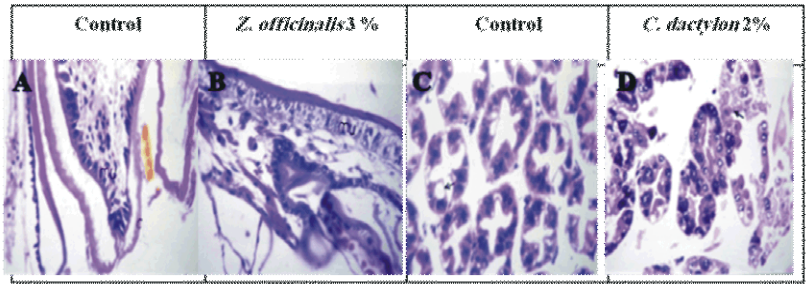


Plate. 1: Stomach of M. Rosenbergii in control (A) and group fed diet incorporated with Z 3% (B) showing normal hisological strucutre of the lining cuticle © mucosal epithelium (mu) lamina propria (sm) and muscularis (ml) Hepatopancreas in control © and group fed diet incorporated with C. Dactylon 2% (D) showing normal histological structure of the tubules lines by different cells (arrow) at 4 weeks from start of feeding (H&E x 80)

most affected cell type by *Z.officinalis* and *C. dactylon* treatment, with the highest significant increase in the group fed with 3% *Z.officinalis* and 4% *C. dactylon* at 4 weeks from the start of feeding (Fig, 2A). In addition, PI and PR of all groups were significantly higher than those of control (Fig, 2B).

Histolopathological Examination: Hepatopancreas and stomach of *Z.officinalis* and *C. dactylon* fed juveniles of all groups showed similar and healthy histological structures without any detrimental effect (Plate, 1).

DISCUSSION

Several studies demonstrated that *Z. officinalis* and *C. dactylon* is beneficial to growth and immune systems in aquatic animals and shrimp [21]. Concerning the effect of herbal plants on growth performance parameters of shrimp and fish. Postlarvae fed on 75 and 100% *Z.officinalis* Artemia enriched had significantly higher FCR and SGR [22]. Pacific white shrimp *Litopenaeus vannamei* juveniles fed with zingrone, one of the constituents isolated from ginger showed significant higher weight gain and feed efficiency [4]. The present study showed that juveniles fed with different doses of *Z. officinalis* and *C. dactylon* showed very significant increase in SGR, CF, WGR, LGR and FCR compared to the control. This increase can be attributed to that herbals and spices active principles in the diets are reported to improve animal performance by stimulating secretion of the digestive enzyme that can result in improvements in digestibility, stimulating the appetite and increasing food consumption and efficiencies. In addition, they shorten the feed transit time which is more prominent in the case of *Z. officinalis*. This reduction in transit time might have a beneficial influence on digestive enzymes and could accelerate the overall digestive process [23]. These results indicate that juveniles fed *Z. officinalis* and *C. dactylon* grow much better compared to previous studies that fed shrimp herbal plant enriched artemia [24] and herbal plant active ingredient [4] and herbal plant extract [25]. This can

be attributed to the active ingredients of Z. *officinalis* [5] and, *C. dactylon* [7] those active ingredients are suspected to enhance appetite, stimulate digestive enzymes and improve the overall digestive process. Similarly, a better growth and production were observed in cultivable fish fed Livol (IHF-1000,which is a herbal growth promoter) [26] and in postlarvae fed Artemia enriched with stresstol [27]. The present study showed that the survival rate among all groups was similar (100%). In a parallel studies, Postlarvae of *P. indicus* fed with the herbal products, stresstol II and stressol I enriched Artemia showed an increase in survival, growth and consumption [27].

Since immunostimulants can increase non-specific immunity by promoting phagocytosis, bactericidal activity, PO activity and respiratory bursts [28]. Rutin extract from *Toona sinensis* administered by injection for *L. vannamei* significantly improved survival rates [29]. The current study showed that Juveniles fed with *Z. officinalis* and *C. dactylon* had significantly higher THC and HV compared to the control. On the same respect, The THC of *M. japonicus* that were fed with MACH at 0.2% and 0.05 showed a significant and rapid increase compared with the control group ($P < 0.05$) over the entire feeding period [30]. The previous increase can be attributed to accelerated maturation of haemocyte precursors in the haematopoietic tissue followed by release of new cells into the circulation [31]. In contrary, There was no significant difference in THCs between shrimp fed the zingerone diets and control shrimp [4].

The present study indicated that small granular hemocytes were the most affected cell type by *Z.officinalis* and *C. dactylon* treatment, with the highest and most significant increase in the group fed with 3% *Z.officinalis* and 4% *C. dactylon* at 4 weeks from the start of feeding. On the same instance, SGH was found to be the most predominant cell in *M. rosenbergii*, representing about 54% from total circulating hemocytes [33]. Versasly, Hyaline hemocytes were the most predominant cell in *P. japonicas* fed with MACH.This difference can be attributed to difference in shrimp species, water salinity and plant species.

In the present study, *Z. officinalis* and *C. dactylon* fed *M. rosenbergii* showed a significant increase in Pahogocytic index (PI) and Pahogocytic rate (PR) compared to the control. Nearly the same results were observed by several authors [33,34].

It is very important to use histological methods to assess the effects of feed on the digestive tract of fish [35]. In our study, *Z.officinalis* and *Cynodon dactylon* fed *M. rosenbergii* juveniles showed almost similar and healthy histological structures of hepatopancreas and stomach without detrimental effect on their structure. On the same respect, *P. semisulcatus* PL fed on mannan oligosaccharides (MOS) showed healthy histological structures and none of the dietary levels of MOS resulted in any detrimental effect on the hepatopancreatic tissue [36].

In conclusion, the present study suggested that *Z.officinalis* and *C. dactylon* can be used as an appetizer, growth promoter and immunostimulant to effectively enhance growth and immune parameters in *M. rosenbergii*.

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