EFFECT OF ZINGIBER OFFICINALIS AND CYANODON DACTYLON AGAINST PH STRESS IN GIANT FRESHWATER PRAWN MACROBRACHIUM ROSENBERGII.

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ABSTRACT

To investigate the ability of Zingiber officinalis (Z. officinalis) and Cyanodon dactylon (C. dactylon) supplementation to antagonize the stressful pH condition on Macrobrachium rosenbergii (M. rosenbergii) juveniles, three groups each of 40 juveniles in two replicates fed diets supplemented with 1.5 % Z. officinalis and 2 % C. dactylon were exposed to pH 9.5 for 5 weeks. Juveniles exposed to high pH and fed with Z. officinalis and C. dactylon had significant increase (p< 0.05) in Average Body Weight (ABW), Average Body Length (ABL), Specific Growth Rate (SGR), Condition Factor (CF), Weight Gain Rate (WGR), Length Gain Rate (LGR) and Feed Conversion Ratio (FCR) compared with those of control along the whole experiment. Juveniles exposed to high pH and fed Z. officinalis and C. dactylon had significantly higher (p< 0.05) Total Hemocyte Count (THC), Hemocyte Viability (HV), Differential Hemocyte Count (DHC), Phagocytic Rate (PR) and Phagocytic Index (PI) as compared with control at 4 and 5 weeks from the start of feeding. Microscopically, hepatopancreas and stomach of Z. officinalis and C. dactylon fed juveniles showed no marked alterations. According to the present study, it could be conducted that Z. officinalis and C. dactylon were effective in controlling the pH stress in M. rosenbergii.

Key words: Herbals – Growth promotor – Immunostimulant – Prawn
1. INTRODUCTION

M. rosenbergii is the most popular prawn species used for commercial farming and has been transported worldwide (New, 2002). At the global level, people have understood the bad effect of chemical products and they are now shifting to natural products (Fauci, 1993). 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 (Citarasu et al., 2001, 2002; Sivaram et al., 2004).

In reared shrimp, the physico-chemical quality of water affect the metabolism, growth, survival and the immune system, therefore the study aimed to investigate this effect of Z. officinalis and C. dactylon on growth performance and immune parameters in M. rosenbergii as well as, their antagonism to overcome high water pH. Water with low (4.6-5.0) and high pH (9.0-9.5) levels have been reported to decrease the THC and PO activity of the fresh water prawn Macrobrachium rosenbergii (Chen et al., 1995).

Z. officinalis is beneficial to growth and immune systems in aquatic animals (Yueh et al., 2012). Ginger rhizomes contain a number of active ingredients (Govindarajan VS., 1982). C. dactylon plant has been used to control various diseases like diabetes, ulcer and diarrhea (Badri and Renu, 2011). The aim of this study was to evaluate the effect of Z. officinalis and C. dactylon, through measuring some growth performance, immune response parameters and histopathological changes of M. rosenbergii stressed juveniles.

2. MATERIALS AND METHODS

2.1. Acclimation of juveniles to 0 ppt salinity:

A total of about 300 M. rosenbergii Juveniles with average body weight 0.3g were obtained from Maryout Project And Company for fish farms, Alexandria, Egypt and stocked into two well prepared cement ponds for one month, ponds were supplied with brackish water 12 ppt salinity that was decreased gradually until reached 0 ppt salinity. Green house ponds were prepared and water parameters were adjusted at Temperature 30ºC, pH 7 and 0 ppt salinity. Juveniles were fed with basal diet (crude protein 30%) (Maryout Company) at rate of 3% body weight (Hien, 1999). Excess feed and excreta were siphoned daily along the acclimation period. Later on, Juveniles were divided into nine groups and stocked in cement ponds at rate of 40 juveniles/pond and left for 3 days for acclimatization.

2.2. Experimental design:

2.2.1. Preparation of experimental diet supplemented with Z. officinalis (Suheyla et al., 2003) and Cyanodon dactylon (Ngan et al., 1988):

Rhizomes of Z. officinalis and whole plant of C. dactylon were shade-dried and ground into fine powder at Agricultural Botany Department, Faculty of Agriculture, Benha University. Three experimental diets were prepared. Fine powdered basal diet was divided
into three portions first portion incorporated with 1.5 % (w/w) Z. officinalis, the second portion mixed with 2 % (w/w) C. dactylon 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 4ºc till use.

2.2.2 Effect of high rearing water pH (9.5) and feeding of Z. officinalis and C. dactylon on growth performance and immune response of M. rosenbergii:

2.2.2.1. Acclimation of juveniles to pH 9.5:

Juveniles were divided into four three and stocked in cement ponds at rate of 40 juveniles/pond and left for 3 days for acclimatization before start of experiment. pH was adjusted at 9.5 along the period of the experiment.

2.2.2.2. Feeding trials:

Three groups were used, one control and two treatment groups in two replicates. The control group and the two treatment groups were fed with control diet and the basal diet supplied with Z. officinalis 1.5 % and C. dactylon 2%, respectively. The treated and control groups received experimental diets for period of five weeks. Feeding amount increased regularly based on body weight measurements every two weeks. Number of dead juveniles was recorded daily in all groups along the experiment.

2.2.2.3. 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.

Body weight was measured using portable electric balance and Body length was measured using graduated ruler. Weight gain rate (WGR), Length gain rate (LGR), Feed conversion ratio (FCR) were calculated according to Bo Liu et al., 2010. Moreover, Specific growth rate (SGR) and condition factor (K) were determined as described by Laird and Needham, 1988.

2.2.2.4. Measurement of immune related parameters:

Hemolymph was collected and divided in two portions one was mixed with Alsever's solution according to FAO, 2002 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 from start of feeding.

THC and HV were measured following the method of Cheng et al., 2003:

\[
\text{THC} = \text{number of counted haemocytes in the 4 corners} \times 2^{\frac{1}{4}} \times 10^4 \times \text{the dilution factor.}
\]

\[
\text{HV (\%)} = (\text{total number of viable cells /total number of cells}) \times 100
\]

DHC was measured according to Wootton and Pipe, 2003; Abdel hameed, 2008. Phagocytic Index (PI) was measured according to Itami et al. [24] with minor modification as following:
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\[ PI = 100 \times \left( \frac{\text{number of haemocytes engulfed beads}}{\text{total number of haemocytes}} \right) \times \left( \frac{\text{number of beads that were engulfed by the haemocytes}}{\text{total number of haemocytes}} \right) \]

Phagocytic rate (PR) was calculated according to Cheng et al., 2003:

\[ PR = \left( \frac{\text{Phagocytic Hemocytes}}{\text{Total Hemocytes}} \right) \times 100. \]

2.2.2.5. Sampling and histopathological examination:

Soon after scarification, samples were taken at 3, 4 and 5 weeks from start of feeding from all groups and fixed in Davidson's Fixative and then subjected to histopathological sectionioning and preparation according to Banchroft et al., 1996.

2.2.2.6. 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 (P<0.05).

3. RESULTS

3.1. Growth parameters:

Growth performance parameters including AW, AL, 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%).
Fig 1 Effect of *Zingiber officinalis* and *Cyanodon dactylon* incorporated diets on average body weight (A), average body length (B), specific growth rate (C), condition factor (D), weight gain rate (E), length gain rate (F) and feed conversion ratio (G) of stressed *M. rosenbergii* for period of 5 weeks.
3.2. **Immune parameters:**

THC and HV of juveniles from all groups were significantly increased compared to the control at 4 and 5 weeks from start of feeding (Table, 1).

Moreover, DHC all groups were significantly increased compared to the control at 4 and 5 weeks from start of feeding. It was clear that small granular hemocytes were the most affected cell type by *Z. officinalis* and *C. dactylon* treatment (Fig. 2A). In addition, PI and PR of all groups were significantly higher than those of control (Fig. 2B).

![Graph A](image1)

![Graph B](image2)

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

3.3. **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:

Recently, several studies were directed to use natural plant products for various activities like anti-stress, growth promotion, appetite stimulation, tonic and immunostimulation, and to have antimicrobial properties in finfish and shrimp (Citarasu et al., 2001). In the present study, M. rosenbergii juveniles efficiently tolerated the exposure to pH (9.5). The results revealed that 1.5% Z. officinalis and 2% C. dactylon fed juveniles tolerate high pH stress effectively and this was expressed in increasing growth performance and immune response of stressed juveniles exposed to pH (9.5) compared to those of control. On the same instance, M. rosenbergii post-larvae fed on Artemia enriched with a herbal medicinal diet tolerated stress efficiently (Citarasu et al., 2002). In addition, the products Stresstol (Citarasu et al., 2002; Cheng and Chen, 2000; Cheng and Jiann, 2008) and Tefroli (Bindhu et al., 2007; Rani, 1999) had a good influence on shrimp post-larvae as the post-larvae fed with the above products were resistant to osmotic pressure, temperature and pH stress. This tolerability can be attributed to the ability of herbal compounds to inhibit the generation of oxygen anions and to scavenge free radicals (Bindhu et al., 2007).

The present study showed that juveniles fed with 1.5% Z. officinalis and 2% C. dactylon had significant increase in ABW, ABL, 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 (Cheng and Jiann, 2008). These results indicated that juveniles fed Z. officinalis and C. dactylon grow much better (El Desouky et al., 2012) compared to previous studies that fed shrimp herbal plant enriched Artemia (Cheng and Chen, 2000) and
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herbal plant active ingredient (Chen et al., 1995) and herbal plant extract (Bindhu et al., 2007). This can be attributed to the active ingredients of Z. officinalis (Govindarajan, 1982) and, C. dactylon (Grzanna et al., 2005; Paranjpe, 2001). 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 fishes fed Livol (IHF-1000, which is a herbal growth promoter) and in postlarvae fed Artemia enriched with stresstol (Venketramalingam et al., 2007). 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 (Citarasu et al., 2002).

Since immunostimulants can increase non-specific immunity by promoting phagocytosis, bactericidal activity, PO activity, and respiratory bursts (Immanuel et al., 2009; Shadakshari, 1993; Jayaprakas and Euphrasia, 1997; Jian and Wu, 2004; Hsieh et al., 2008). Rutin extract from Toona sinensis administered by injection for L. vannamei significantly improved survival rates (Jayaprakas and Euphrasia, 1997). The current study showed that Juveniles fed with 1.5 % Z. officinalis and 2 % 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 (Amel et al., 2010). 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 (Amel et al., 2010; Cheng et al., 2004). In contrary, there was no significant difference in THCs between shrimp fed the zingerone diets and control shrimp (Chen et al., 1995).

The present study indicated that small granular hemocytes were the most affected cell type by Z. officinalis and C. dactylon treatment. On the same instance, SGH was found to be the most predominant cell in M. rosenbergii, representing about 54% from total circulating hemocytes (Sequeira et al., 1996). Versusly, Hyaline hemocytes were the most predominant cell in P. japonicas fed with MACH (Amel et al., 2010) and p. japonicas injected with 1, 3-glucan (Cheng et al., 2004). This difference can be attributed to difference in shrimp species, water salinity and plant species.

In the present study, 1.5 % Z. officinalis and 2 % Cynodon dactylon fed M. rosenbergii showed a significant increase in Phagocytic index (PI) and Phagocytic rate (PR) compared to the control. Nearly the same results were observed by several authors (Amel et al., 2010; Sequeira et al., 1996; Sörderhäll et al., 2003).

It is very important to use histological methods to assess the effects of feed on the digestive tract of fish Božidar et al., 2011; Miriam and Menon, 2005). In our study, Z. officinalis and C. 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 (Gene et al., 2007).

In conclusion, the present study documents that Z. officinalis and C. dactylon increase tolerability to pH stress in M. rosenbergii and can be used as an appetizer, growth promoter and immunostimulant to effectively enhance growth and immunity parameters.
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oligosaccharide on growth, body composition and hepatopancreas histology of
Table (1) Effect of *Zingiber officinalis* and *Cyanodon dactylon* incorporated diets on Total Hemocyte Count (10⁶/ml) and Hemocyte Viability (%) of stressed *M. rosenbergii* at 4 and 5 weeks.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Total Hemocyte Count</th>
<th>Hemocyte Viability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 (w₁)</td>
<td>5 (w₁)</td>
</tr>
<tr>
<td>Control</td>
<td>5.7±0.057d</td>
<td>3.9±0.057d</td>
</tr>
<tr>
<td><em>Z. officinalis 1.5%</em></td>
<td>15.9±0.057a</td>
<td>22.2±0.057a</td>
</tr>
<tr>
<td><em>C. dactylon 2%</em></td>
<td>10.2±0.057c</td>
<td>10.2±0.057c</td>
</tr>
</tbody>
</table>

Mean values (± SE) at the same exposure time with different superscript letters are significantly different at level of P< 0.05.
تأثير الجنزبيل والنجيلة البلدية لمقاومة إجهاد الأس الهيدروجيني على جمبرى المياه العذبة

هيثم العيد، منال عباس، امل العسلى، أيمن عبد الجواد، عادل شاهين
كلية الطب البيطري، جامعتها، جمهورية مصر العربية.

لدراسة قدرة الجنزبيل ونجيلة البلدية على مواجهة الأس الهيدروجيني كعامل ضاغط على جمبرى المياه العذبة. ثلاث مجموعات كل منها تتكون من 40 زريعة تم تغذيتها على الجنزبيل بتتركز 1.5% ونجلة البلدية بتتركز 2% وتم تعرضها لأس هيدروجيني 9.5 لمدة خمس أسابيع.

وقد أظهرت جميع المجموعات زيادة ملحوظة في معدلات النمو والاستجاب الاستجابة للمناعية مقارنة بالمجموعات الحاكمة. وأوضحت الصورة الهيستولوجية للمجموعات المغذاة على كل من الجنزبيل والنجلية البلدية عدم وجود تغيرات ملحوظة في التركيب الهيستولوجي للمعدة والهيباتوبكرياس.