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Effect of stocking density on growth performance, digestive enzyme activities, and nonspecific immune parameters of *Palaemonetes sinensis*

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ABSTRACT

Palaemonetes sinensis is a new breed of shrimp with great potential for aquaculture, which has been confirmed in our previous production tests. However, there are limited reports about this species and its biological information is scarce. This study describes the effect of stocking density on the growth, digestive enzyme activities, and nonspecific immunity of *P. sinensis* with an initial average body weight was 0.25 ± 0.02 g. Groups of shrimps were reared at four different initial densities (2.5, 5, 10, and 20 individuals·L^{-1}). After 30 days of culture, the results indicated that the final body weight, weight gain, and specific growth rate were higher in shrimps grown in groups of 10 individuals·L^{-1} than other groups, but the survival rates of these shrimp were significantly lower than those reared in group of 2.5 or 5 individuals·L^{-1}. The trypsin, amylase, and lipase activities of shrimp significantly decreased with increase in stocking density. Nonspecific immune indicators decreased significantly with increase in density, but there were no significant differences between the 2.5 and 5 individuals·L^{-1} groups in terms of the total haemocyte count (THC), phenoloxidase activity (PO), lysozyme (LZM), catalase (CAT), and superoxide dismutase (SOD). These results suggest that increasing the stocking density from 2.5 to 5 individuals·L^{-1} did not affect any of the detected indicators of *P. sinensis*, but there are shelter in farming mode is better for culture of *P. sinensis* up to 10 individuals L^{-1}.

1. Introduction

The Liao river shrimp (*Palaemonetes sinensis*) is a native shrimp species with great potential for aquaculture, based on promising results obtained from experimental culture trials in North China. There are several environmental and genetic benefits for using native shrimp species rather than exotic ones, requiring new introductions of this species. The indigenous species *P. sinensis* is a good alternative for culture, and it can be used in a variety of rearing strategies, including polyculture, to increase unit yield, but its biological characteristics are not clear. Very few studies have investigated the biological features of this species. *P. sinensis* is an endemic species with a large geographic distribution in the middle and lower reaches of the Yangtze River and in North China [1]. *P. sinensis* can consider doing model creatures as its hold eggs breeding habits, and its meat has a firm texture and pronounced flavour as well as good nutritional value.

Temperature and food supply are the main external factors that influence the growth of crustaceans [2]. However, the growth rate of the freshwater shrimp *Macrobrachium amazonicum* is dependent on stocking density [3,4]. Other factors such as sexual maturity and population structure also interfere with the growth of freshwater prawns. In the past several decades, shrimp culture in China has suffered from problems linked to deteriorated pond environment due to poor management. Therefore, enhancing the shrimp's immunity is of primary concern for farming practice. Shrimp do not have immunoglobulin (Ig), and they resist diseases and viruses using another approach, i.e., nonspecific immunity. Nonspecific immunity factors such as the total haemocyte count (THC), phenoloxidase activity (PO), respiratory burst (O_2^-), superoxide dismutase (SOD) activity, phagocytic activity, and lysozyme (LZM) activity play important roles in the immune response and disease resistance of shrimp [5,6]. Stocking density is another important factor in shrimp culture, and significantly affects shrimp growth, survival, and yield [7]. There is little information about *P. sinensis* cultures, and few studies have investigated the effects of stocking

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density on growth performance and nonspecific immunity response. Therefore, in the present study we evaluated the influence of stocking density on the growth, survival rate, and nonspecific immune factors of *P. sinensis* reared in indoor glass tanks.

2. Materials and methods

2.1. Shrimp rearing

P. sinensis were supplied by the Research & Development Center, Panjin Guanghe Crab Industry Co. Ltd. Upon arrival, the shrimp were acclimated to laboratory conditions for 14 days in indoor fibre-reinforced plastic tanks and fed a commercial diet (Liuhe, Qingdao, China). The laboratory conditions during the acclimation period were similar to those at the initiation of the experiment.

A total of four treatments were administered. At the beginning, a total of 1686 shrimp were randomly assigned to four groups at stocking densities of 2.5 (D I), 5 (D II), 10 (D III), and 20 (D IV) individuals·L^{-1}. There were six replicates for 2.5 individuals·L^{-1} and three replicates in each of the remaining groups. The tanks were aerated by a single airstone to maintain the dissolved oxygen at 5–7 mg L^{-1}. The shrimp were fed twice daily (at 07:00 and 18:00 h) at a ratio of 8% of their body weight. This amount was close to the maximal daily rations consumed by the shrimp during the acclimation period. During the experimental period, the water temperature was maintained at 17 °C and the pH at 8.0–8.5.

2.2. Growth and survival

To monitor growth, at the end of the 30-day feeding trial, all shrimp were sampled from each tank, weighed, and the remaining shrimp in the tanks were counted to determine survival. Growth was measured as the percentage of body weight gain per surviving shrimp in each aquarium, calculated as follows: weight gain rate (WGR, %) = [(final weight − initial weight)/initial weight] × 100; specific growth rate (%/d) = (LN final weight − LN initial weight) × 100/days; survival rate (%) = final shrimp number × 100/initial shrimp number.

2.3. Digestive enzyme activities assays

At the end of the experiment, the shrimps were anesthetized in slurry ice, and their trypsin, amylase, and lipase activities were measured using the assay kit provided by Jiancheng Bioengineering Institute (Nanjing, China), according to the manufacturer's instructions. The hepatopancreas and intestines were weighed and homogenized at 4 °C in sterile saline solution. Shrimp (n = 5) in each tank of four treatment groups were washed with sterile distilled water, kept individually in 1.5-mL eppendorf tubes, and stored at −20 °C until digestive enzymatic analysis performed in two weeks.

2.4. Nonspecific immune response assays

After the final weighing, each shrimp was blotted dry, and their haemolymph was collected from the ventral region of the cephalothorax with a 1-mL syringe, which was rinsed with anticoagulant (4.2 g L^{-1} NaCl, 8 g L^{-1} trisodium citrate, 0.55 g mL^{-1} citric acid, 20.5 g L^{-1} D-glucose; pH 7.55). Anticoagulant and haemolymph were mixed at a ratio of 1:1, and 25 μL of this mixture was used for the THC assay, 200 μL was used to assess phagocytic activity. Then, 100 μL of haemolymph was centrifuged at 800 × *g* for 10 min at 4 °C, and the supernatant was used to estimate the PO, SOD, LZM, acid phosphatase (ACP), and catalase (CAT) activity.

2.4.1. THC measurement

Haemolymph (100 mL) was withdrawn and mixed with 900 mL of anticoagulant solution [8]. A drop of diluted haemolymph was placed on a haemocytometer to measure the THC using an inverted phase-contrast microscope (Olympus IX-71, Tokyo, Japan). Treatments were measured in triplicate for each sample.

2.4.2. Phagocytic activity assay

Phagocytic activity was analysed according to the method described by Liu and Chen [9]. Phagocytic activity was defined as the phagocytic rate (PR) of shrimps against *Escherichia coli*, as follows: PR = [(phagocytic haemocytes)/(total haemocytes)] × 100%.

2.4.3. PO activity assay

PO activity was measured as described by Ashida (1971) [10]. Briefly, 10 μL of serum, 300 μL phosphate buffer (0.1 M, pH 6.0), and 10 μL of dihydroxyphenylalanine solution (0.01 M) were added to 96-well microtiter plates, and the absorbance (optical density at 490 nm [OD_{490}]) was determined every 2 min. An increase of 0.001/min in the OD_{490} was regarded as 1 unit of activity.

2.4.4. SOD, LZM, ACP, and CAT activity assays

The pooled serum was stored at −80 °C before analysis of the SOD, LZM, ACP, and CAT activities. The SOD, LZM, ACP, and CAT activities were assayed using the corresponding detection kits (Nanjing Jiancheng Biological Product, China) according to the manufacturer's guidelines.

2.5. Statistical analysis

Data were expressed as mean ± standard deviation (SD). All data were analysed by one-way analysis of variance (ANOVA) using SPSS 19.0 statistical software. When the ANOVA detected significant differences between the treatment groups, multiple comparisons were made with Duncan's new multiple range tests, and statistical significance was set at $P < 0.05$.

3. Results

3.1. Growth and survival

At the end of the 30-day feeding trial, the final body weight, weight gains, and specific growth rate were higher in the 10 individuals·L^{-1} group than the other groups ($P < 0.05$), but there were no significant differences between the 2.5 and 5 individuals·L^{-1} groups (Table 1). After raising shrimp at different stocking densities for 30 days, the survival rate exhibited a downward trend, and the 10 and 20 individuals·L^{-1} groups significantly differed from the 2.5 and 5 individuals·L^{-1} groups ($P < 0.05$; Table 1).

3.2. Digestive enzyme activity

The trypsin and amylase activities of shrimp significantly decreased

Table 1
Final body weight (FBW), weight gain (WG), specific growth rate (SGR) and survival rate of *Palaemonetes sinensis* after the 30-day feeding trial.

	D I	D II	D III	D IV
FBW (g)	0.27 ± 0.01^{b}	0.27 ± 0.01^{b}	0.35 ± 0.01^{a}	0.24 ± 0.01^{b}
WG (%)	7.35 ± 1.17^{b}	6.87 ± 0.76^{b}	13.06 ± 0.61^{a}	3.38 ± 0.68^{c}
SGR (%/-d)	0.23 ± 0.02^{b}	0.20 ± 0.01^{b}	1.21 ± 0.01^{a}	0.10 ± 0.01^{c}
Survival rate (%)	72.16 ± 2.70^{a}	76.67 ± 3.52^{a}	50.67 ± 3.52^{b}	43.00 ± 0.67^{b}

Values are the means ± SD, and values in the same row with different letters (a,b and c) are significantly different ($P < 0.05$).