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Short sequence report

Effect of dissolved oxygen on immune parameters of the white shrimp *Litopenaeus vannamei*

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Dissolved oxygen (DO) is one of the most important environmental stress factors in aquaculture and is affected by many environmental factors, such as the sudden change or death of the dominant population of phytoplankton, large reproduction of zooplankton in the pond, and decomposition of accumulated organic matter including unconsumed food and faeces, which will lead to sharp decreases of DO. Thus, decreases in DO are a common hazard in shrimp culture.

Hypoxia can affect the survival and growth [1–3], respiration [4–7], hemolymph osmotic pressure [8,9], and be a cause of mortality [10,11] in shrimp. The effect of hypoxia has been reported to reduce the total haemocyte count (THC) of crustaceans and lead to an enhanced sensitivity to pathogens [12,13]. The aim of the present work was to study the effect of dissolved oxygen on immune parameters of *Litopenaeus vannamei*, to gain more information on the mechanism of the shrimp immunological regulation and also provide a scientific basis for water quality regulation in shrimp culture.

L. vannamei adults used in this experiment were obtained from a commercial farm in Yinghai, Qingdao. The average body length was about 8.5 ± 0.5 cm. The shrimps were acclimated in tanks (30 cm × 40 cm × 50 cm) containing aerated water (salinity 30%, pH 8.0) with an air-lift at 18 ± 0.5 °C for 8–10 days prior to experimentation. Half of the water in each tank was renewed twice daily. During the acclimation period, the shrimps were fed a formulated shrimp diet daily. Before the experiment the shrimps were not fed for 48 h.

The experimental system comprised hermetic service tanks and the test tanks. The volume of the service tanks was 1000 l and that of test tanks was 80 l. The two tanks were connected with soft pipe through which the water of the service tanks could flow into the test tanks at a rate of 20 l h^{-1}. Four DO levels of

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2.0,3.5,5.5 and 7.5 mg l^{-1} were established. The desired concentration of DO in each test tank was established by bubbling nitrogen gas and air into the water of the service tank. The test groups comprised 20 shrimps, respectively. During the experiment, DO was monitored with a DO meter (YSI-556MPS model) every 4 h and varied about 2.05 ± 0.12, 3.53 ± 0.21, 5.48 ± 0.10 and 7.48 ± 0.15 mg l^{-1}, respectively, and no shrimps died. For each treatment, there were three replicate groups. Three shrimps were sampled randomly from each group at 0, 4, 8, 12, 24 and 48 h.

After exposure in each treatment, haemolymph was withdrawn from the ventral sinus of each shrimp into a 1.0 ml syringe containing an equal volume of anticoagulant solution (450 mM NaCl, 10 mM KCl, 10 mM EDTA–Na_2, 10 mM HEPES, pH 7.45). Samples of the haemolymph for the haemocyte count were mixed in a centrifuge tube without centrifuging and used immediately. Other samples were held in a refrigerator (4 °C) for 24 h, centrifuged (3000 rpm) at 4 °C for 15 min, and the blue plasma was separated for measurements of other immune parameters.

Total haemocyte counts (THC) were performed in a haemocytometer using an Olympus phase contrast microscope.

Phenoloxidase activity was measured spectrophotometrically by recording the formation of dopachrome from L-dihydroxyphenylalanine (L-DOPA) as previously described [14].

Bacteriolytic activity was measured using *Micrococcus lysodeikticus* (Sigma) and antibacterial activities were measured with *Vibrio parahaemolyticus* and *Vibrio harveyi*, according to described methods [15]. *V. parahaemolyticus* and *V. harveyi* were cultured on 211E-beveled solid substrates for 18–20 h at 30 °C, washed with 0.1 M phosphate buffer (pH 6.4) and the bacteria suspended in the buffer to $O.D._{570\ nm} = 0.3$.

Each bacterial suspension (3 ml) was mixed with 50 μl plasma in tubes in ice water (0 °C) and the optical density at 570 nm (A_0) was measured. The tubes were then transferred to a water bath at 37 °C for 30 min, then returned to ice water (0 °C) for 10 min to stop the reaction and the optical density at 570 nm (A) was measured again.

The antibacterial activity was calculated as follows:

$$U_a^2 = (A_0 - A)/A$$

The bacteriolytic activity was calculated as follows:

$$U_b = (A_0 - A)/A$$

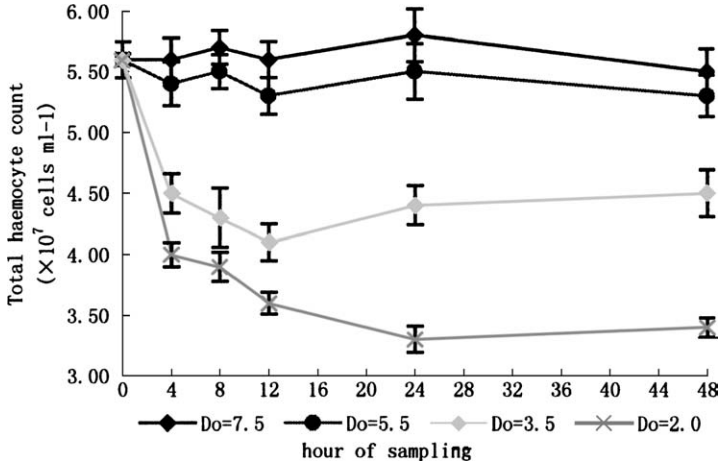

Fig. 1. Effect of dissolved oxygen on the haemocyte count of *L. vannamei*. The data represent means ± S.D. ($n = 9$).