Normal immuno-physiological parameters in black tiger shrimp (*Penaeus monodon*) and the effects of environmental stress and diseases

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KEY WORDS: immuno-physiology, blood parameter, immune system, black tiger shrimp, *Penaeus monodon*, environmental stress

INTRODUCTION

The immune system of crustaceans responds to pathogens and foreign bodies through cellular and humoral factors. A majority of the response is non-specific. It has been established that three types of blood cells (hyaline, semi-granular and large granular hemocytes) and fixed phagocytes have the important role in the immune process. The important humoral factors are the pro-phenoloxidase activating system and lectin, which enhance the phagocytic function of blood cells. This study reported the normal immuno-physiological parameters in black tiger shrimp, effect of environmental conditions and the occurrence of diseases on blood component and immune function.

MATERIAL AND METHODS

Healthy shrimp with a weight between 10-20 g were obtained from shrimp farms in Amphoe Ranot, Songkhla Province (East Coast of Southern Thailand and Amphoe Langu, Satun Province (West Coast of Southern Thailand)(earthen pond samples). Samples were examined under light microscope for parasitic infestation. Microbiological method were performed for bacterial infection and PCR analysis was applied for WSV contamination. Negative samples were used in this studies. Collected samples were based on sex, size (<20 g and >20 g) and molting cycle (postmolt: A, B, intermolt: C and premolt: D) (Smith and Doll³). Blood was immediately withdrawal to determined normal physiological parameters i.e. total hemocyte count (THC), blood glucose and phenoloxidase activity (PO activity). Samples were maintained at the Aquatic Animal Health Research Center, Prince of Songkla University, Songkhla, Thailand and reared in glass tanks with continuous aeration for 7 days prior to further experiment (laboratory samples). The shrimp were divided into 4 groups to determine 1) the normal physiological parameters 2) immuno-

physiology based on sex, size and molting cycle 3) effect of water temperature (25 and 30 °C) and dissolved oxygen (0.9-1.2 and 5.0-5.8 mg/l) on immuno-physiological function and 4) effect of diseases (luminescent bacteria and virus) on immuno-physiological functions and blood components.

RESULTS

I Normal immuno-physiological and blood parameters.

The hemocyte count for the samples from earthen ponds is significantly higher than the laboratory samples. Significant difference was also record for PO activity for farm and laboratory samples. However, the level of blood sugar in laboratory samples was significantly higher than those in the earthen ponds samples (Table 1).

Table 1 Immunological and blood parameters of black tiger shrimp from the laboratory and earthen ponds.

Parameters	Laboratory	Earthen ponds
THC ($\times 10^4$ cell/mm ³)	3.07 $\pm$ 1.91(n=519) ^a (0.35-10.25) ¹	6.49 $\pm$ 2.33(n=246) ^b (0.42-14.30) ¹
PO activity (unit/min/mg protein)	217.84 $\pm$ 161.99(n=233) ^a (15.15-1,142.85) ¹	335.18 $\pm$ 106.29(n=130) ^b (99.24-655.96) ¹
Blood glucose (mg%)	53.87 $\pm$ 54.84(n=44) ^b (1.91-228.03) ¹	32.91 $\pm$ 27.95(n=130) ^a (8.24-165.88) ¹

n = number of analyzed samples

¹ = range

Mean within row not sharing a common superscript is significantly different (p<0.05)

II Sex, size and molting cycle differences in immuno-physiological parameters.

THC and PO activity of all samples did not sex and size differences. However, a difference was found in various stages of molting. The THC recorded the peak in the premolt stages and declined to the lowest in postmolt stage. PO activity in the hemocytes was highest during intermolt stages and lowest during postmolt stage (Table 2 and 3). However, there was no statistical difference in

blood sugar levels between sex, size and molting cycle.

Table 2 THC of black tiger shrimp from the earthen pond and the laboratory based on molting cycle

Sources of Samples	Molting cycle		
	A,B	C	D
Ranot	4.95±1.02 ^{ns}	5.05±1.52 ^{ns}	5.76±1.53 ^{ns}
Songkhla	(n=12)	(n=28)	(n=20)
Langu	5.56±0.90 ^a	6.91±1.89 ^{ab}	7.59±2.30 ^c
Satun	(n=11)	(n=24)	(n=27)
Laboratory	1.36±1.10 ^{ns}	1.60±1.48 ^{ns}	1.56±0.90 ^{ns}
	(n=3)	(n=25)	(n=22)

THC= $\times 10^4$ cell/mm³ ns = not significant

n = number of analyzed samples

Mean within row not sharing a common superscript is significantly different ($p < 0.05$)

Table 3 PO activity of black tiger shrimp from the earthen pond and the laboratory based on molting cycle

Sources of Samples	Molting cycle		
	A,B	C	D
Ranot	374.56±368.54 ^{ns}	368.54±113.38 ^{ns}	393.53±104.31 ^{ns}
Songkhla	(n=5)	(n=32)	(n=26)
Langu	280.97±65.96 ^a	299.23±91.68 ^{ab}	293.64±74.09 ^b
Satun	(n=9)	(n=36)	(n=21)
Laboratory	101.82±49.03 ^{ns}	89.32±41.12 ^{ns}	66.78±47.36 ^{ns}
	(n=7)	(n=21)	(n=22)

PO activity = unit/min/mg prot.

n = number of analyzed samples ns = not significant

Mean within row not sharing a common superscript is significantly different ($p < 0.05$)

III Effect of water temperature and dissolved oxygen on immuno-physiological function

Low concentrations of DO (0.9-1.2 mg/l) and temperature (25 °C) resulted in a reduction of THC with 2.75 ± 1.40 and $2.03 \pm 0.92 \times 10^4$ cell/mm³, respectively when compared with shrimp under normal DO (5.0-5.8 mg/l) and high water temperature (30 °C) with 3.58 ± 2.03 and $3.15 \pm 1.24 \times 10^4$ cell/mm³, respectively. Similar trend was recorded for PO activity with 249.38 ± 182.72 and 225.88 ± 138.95 unit/min/mg protein for low DO and temperature and 458.23 ± 241.11 and 365.49 ± 217.12 unit/min/mg protein, respectively.

IV Effect of diseases on immuno-physiological function and blood composition

Black tiger shrimp infected with luminescent bacteria (*Vibrio harveyi*), white spot virus (systemic ectodermal and mesodermal baculovirus: SEMBV) and yellow head virus (YHV) showed significant reductions in immunological parameters including THC with 3.08 ± 1.06 , 1.26 ± 0.42 , 0.54 ± 0.22 and $0.82 \pm 0.18 \times 10^4$ cell/mm³ for control, *V. harveyi*, SEMBV and YHV infected and PO activity with 439.71 ± 60.76 , 296.52 ± 174.46 , 141.23 ± 96.84 and 197.10 ± 102.61 unit/min/mg protein, respectively.

DISCUSSION

The normal ranges of immuno-physiological parameters were determined in black tiger shrimp collected from the farms and wet laboratory and showed wide variations depending on rearing conditions. In general, the "basal" immuno-physiological and blood parameters of shrimps collected from earthen ponds are higher than samples from laboratory. Our findings were in line with those of Vargas-Albores⁴ who reported that PO activity and serum protein in *Penaeus californiensis* varied with the temperature and salinity. We concluded that external parameters such as lower water temperature could influence blood parameters and immuno-physiological functions. Various stages of molting cycle also affected immuno-physiological functions. THC and PO activities from shrimps in premolt or D-stage were higher than A, B and C-stage. The results agreed with Le *et al.*¹⁾ in that THC and PO activity was decreased during intermolt stage and increased during premolt stage. This indicated that shrimp in postmolt stage had lower immunity and could be less resistant to infection with pathogen such as luminescent bacteria, SEMBV and YHV. Furthermore, the reduction of water temperature and DO could also cause a decrease in immuno-physiological functions. We found that THC and PO activities decreased when the shrimp are reared in lower temperature and DO condition. Matinez-Palacios *et al.*²⁾ found that body weight and respiratory rate of *P. vannamei* decreased when DO and temperature is low. That could be related to a decrease in immuno-physiological functions. Our results also showed the reduction of immuno-physiological parameters in pathogen infected shrimps and clearly showed that viral infection had a greater effect on the immune system than did bacterial infection.

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