LIPOPOLYSAHCARIDS INJECTED ON HAEMOLYPMH OF REDCLAW (*Cherax quadricarinatus*): A STUDY IN THE IMMUNE SYSTEM

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ABSTRACT

The aim of this study was to study about LPS induction from Aeromonas hydrophyla, to improve innate immunity of red claw (Cherax quadricarinatus). There were any test at the hemolymph had been performed to reach the direction, that were activation of prophenoloxidase (proPO) and fagocytosis activity.

Material used was Red claw 40 species. The method used was experimental method with Complete Randomized Design consisted of 4 kinds of treatment: P1 (without injection of LPS), P2 (LPS: 0.5 mg/ml), P3 (LPS: 1.0 mg/ml), and P4 (LPS: 1.5 mg/ml). Each treatment was performed triplicate. Replication performed was the taking of hemolymph sample at 2nd and 3rd week after injection of LPS. Research parameters evaluated were activation of prophenoloxidase (proPO), phagocytosis activity, and gene characterization responsible at the immune system. Data, was analyzed by Analysis of Varians, and was continued by BNT test.

It could be conclude that induction of LPS by injection at red claw species was not significantly difference (P>0.05) against activation of proPO, was significantly difference with phagocytosis activity (P<0.05). injection of LPS at 1 mg/ml concentration or 1.5 mg/ml showed the highest phagocytosis activity.

Keywords : Red claw, immune system, phagocytosis, proPO

ABSTRAK

Penelitian ini bertujuan untuk mengkaji induksi LPS dari bakteri Aeromonas hydrophila *untuk meningkatkan sistem kekebalan innate lobster air tawar jenis red claw* (Cherax quadricarinatus).

Beberapa uji dilakukan terhadap hemolymph : sistem pengaktifan ProPO dan aktivitas fagositosis, Digunakan 40 ekor red claw, dengan metode eksperimental, rancangan RAL, terdiri dari 4 perlakuan : P1 (tanpa injeksi LPS), P2 (LPS: 0.5 mg/ml), P3 (LPS: 1.0 mg/ml), dan P4 (LPS: 1.5 mg/ml). Perlakuan diulang 3 kali, yaitu 1 minggu, 2 minggu dan 3 minggu pengambilan sampel hemolymph setelah injeksi LPS. Parameter yang diukur adalah aktivitas profenoloksidase (ProPO), fagositosis dan karakteristik gen penanggung jawab sistem imun. Data dianalisis dengan Anava, dan diuji lanjut dengan uji BNT.

Disimpulkan bahwa induksi LPS tidak berpengaruh nyata(P>0,05) terhadap pengaktifan ProPO, tetapi berpengaruh nyata (P<0,05) terhadap aktivitas fagositosis, dengan aktivitas tertinggi pada level 1 mg/ml atau 1,5 mg/ml.

Kata Kunci : *Red claw*, sistem imun, fagositosis, profenoloksidase (proPO)

INTRODUCTION

Red Claw (Cherax quadricarinatus) is one of important fresh water organism to be cultured in Indonesia. It has many superior quality compared to the other organism, such as its high tolerancy to the low quality of water and its ability to be cultured in a high density (Jones, C.M., 2000; Hernandez-Vergera et al, 2003; Muzinic et al, 2004). However, the appearance of diseases caused the decreased of its production. The increasing of red claw culture in Indonesia could cause the appearance of various diseases problems. Aeromonas hydrophila is one of bacteria contaminating red claw. It could cause hemorrhagic septicemia in many fresh water fish and lobster species.

Solution to manage this problem is by the usage of chemical substances. However, the use of this substances is not solved the problem. On the contrary, it could affect the environment. On the other hand, the use of vaccine could not used on crustacean, because this kind of species does not have adaptive immunity on their body. However, even they do not have adaptive immunity on their body, crustacean had an innate immunity that could be used to destroy foreign material coming to their body.

Immune respon of red claw is understood in this study which use LPS (Lipopolysahcaride) from negative bacteria that could improve complementary component as one of innate immunity factor and stimulate fagocytosis activity of macrophage (Lunde and Robertsen, 1997). Mixture of crustacean hemocyte with LPS was evaluated in vitro and obtained degranulation of granulocyte cell and activate ProPO.

MATERIAL AND METHOD

Material

Material used in this study were 40 organism of red claw at homogenous age and body weight. It was separated to 4 units of aquarium, each of it was contained 10 organism.

Method

Method used in this study was experimental by using completed randomized design (RAL) and consist of 4 types of treatments in different concentration of LPS injection. The concentrations were: 0 mg.ml LPS, 0.5 mg/ml LPS, 1.0 mg/ml LPS, and 1.5 mg/ml LPS. Each of treatment was performed triplicate at the haemolymph of the organism. Samples were taken at the 2nd and 3rd week after injection of LPS. Sample was taken on 3 organisms in each aquarium and haemolypmh was taken from 5th of walking leg in different concentration of LPS injection. The concentrations were: 0 mg.ml LPS, 0.5 mg/ml LPS, 1.0 mg/ml LPS, and 1.5 mg/ml LPS. Each of treatment was performed triplicate at the haemolymph of the organism. Samples were taken at the 2nd and 3rd week after injection of LPS. Sample was taken on 3 organisms in each aquarium and haemolypmh was taken from 5th of walking leg.

Activation of ProPO

Prophenoloxidase system was analyzed by using method performed by Hernandez-Lopez et al (1996). Haemolymp was centrifuged at 300 g at 4^{0}C for 10 minutes. Then, the supernatant was removed from the sample. Pellet of the centrifuged sample, then, was cleaned and resuspend on 1 ml of cacodylate-citrate buffer. After it was incubated, 100 µl L-DOPA and 800 µl of cacodylate buffer was added and incubated for 5 minutes. Samples, then, were evaluated by using optical density 490 nm.

Fagocytosis activity

Fagocytosis activity was evaluated by a protocols developed by Selvin et al (2004). A mixture of 10 µl inactivated bacteria and suspense with PBS were added on 100 µl of haemolypmh. The mixture was

placed on a cover slip and incubated at 20^0C for 30 minutes. Cell, then, was fixated by using methanol and colored with may-Grunwald-Giemsa for 15 minutes and observed under microscope at 1000 x of magnification.

Data Analysis

Data were analyzed by using analysis of variant. If the result of analysis is significantly difference, it was continued by BNT test to know the differences among treatment.

RESULT AND DISCUSSION

Activation of proPO

Based on the result, absorbance rate of proPO haemolymph of Red claw was ranging at 0.009733 to 0.0587. The lowest absorbent rate was shown by a treatment using injection of LPS concentration at 0 mg/ml, while the highest absorbent rate was shown by treatment using LPS injection at 1.5 mg/ml (Table 1). The description of proPO absorbent rate of red claw haemolypmh could be seen at Figure 1.

Treatment using concentration of 0 mg/ml showed absorbent rate at 0.0026, treatment of LPS injection using concentration 0.5 mg/ml showed absorbent rate 0.0286, treatment LPS injection using concentration 1 mg/ml showed an absorbent rate 0.0081, and the LPS injection using concentration 1.5 mg/ml was obtained absorbent rate 0.0074.

Table 1. Mean of absorben rate (±SD) LPS injection at various concentration on red claw haemolymph.

Tretament	Replication			Total	Mean	(±)SD
	1	2	3			
P1 (0 mg/ml LPS)	0.0026	0.0045	0.0221	0.0292	0.009733	0.01
P2 (0.5 mg/ml LPS)	0.0286	0.0189	0.037	0.0845	0.028167	0.01
P3 (1 mg/ml LPS)	0.0081	0.0999	0.0331	0.1411	0.047033	0.05
P4 (1.5 mg/ml LPS)	0.0074	0.1654	0.0033	0.1761	0.0587	0.09

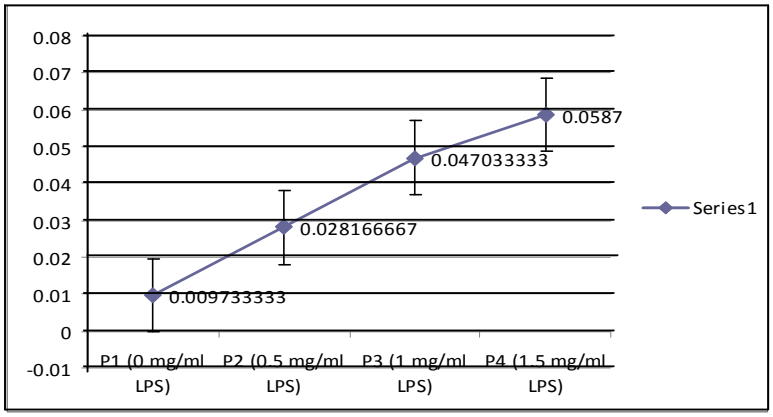

Figure 1. Description of mean of absorben rate (±SD) LPS injection at various concentration on red claw haemolymph

Cellular immune systems are fagocytosis, encapsulation, and cytotoxicity. Red claw had only innate immunity which is able to works effectively to detect and destroy the foreign protein including pathogenic microorganisms. Red claw does not have leukocyte, but they have haemocyte which is important immune system similar to leukocyte. Beside the existence of haemocyte, the existence of serine proteinase such activation of proPO involved in the activation of innate immunity reaction.

Absorbent rate at the 3rd week after injection of LPS was higher than the 2nd week showed that there is indicate of proPO activation increase. Activation of proPO was caused by the induction of LPS as an immunostimulant. LPS will be bound to the receptor inside of haemocyte surface. If there is infection of organism, haemocyte will faced it by degranulates and release important molecules in the mechanism of innate immunity. It is similar with statement of Jussila (1997) that releasing of inactive proPO to the haemolymph farom granulocyte could be initiated by LPS from negative gram bacteria.

At the activation of proPO, zimogen proPO is activated by serine proteinase became PO, thus PO activate some molecules. It caused many defense mechanism on the body. It is similar with a study performed by Hastuti (2004) whether activation of proPO will activate any defense mechanisms such as encapsulation and melanization of pathogenis cell.

Fagocytosis activity

Based on the result, percentage of fagocytosis was ranging from 29.08667 to 40.19333 (Table 2). The highest result was shown by treatment using injection of LPS at concentration 1.5 mg/ml (P1), while the lowest result was shown by treatment using injection of LPS at concentration 0 mg/ml. Treatment using injection of LPS at concentration 0.5 mg/ml and 1 mg/ml showed a percentage of fagocytosis percent at 33.38667 and 37.24, respectively.

There was a significance difference on the injection of LPS among treatment against Aeromonas hydrophyla to the fagocytosis of Red claw. It could be concluded form the result that LPS injected on the haemolypmh of Red claw could functioned as immunostimulant. It is synergize with a statement that immunostimulant is a substance used to increase the defense system activity against pathogenic substances, beside that increase the survival rate of cultured organism when it is attacked by dangerous pathogen. Immunostimulant could be formed from live

Table 2. Percentage of fagocytosis (±SD) LPS injection at various concentrations on red claw haemolymph.

Tretament	Replication			Total	Mean	(±)SD
	1	2	3			
P1 (0 mg/ml LPS)	29.33	27.27	30.66	87.26	29.08667	1.71
P2 (0.5 mg/ml LPS)	36.27	32.58	31.31	100.16	33.38667	2.58
P3 (1 mg/ml LPS)	34.45	37.46	39.81	111.72	37.24	2.69
P4 (1.5 mg/ml LPS)	40.39	42.13	38.06	120.58	40.19333	2.04

bacteria, inactivated bacteria (bacterin or bacteria antigene), glucans, peptidoglican, and lipopolysahcarides. The use of live bacteria had been inactivated similar with the simple vaccine forming concept. Effect of LPS as immunostimulant was studied, which reported that LPS is one of surface receptor important for the engulfing of antigen.

CONCLUSION

It could be concluded from the result that injection of LPS on the haemolymph of Red claw. Mechanism of proPO activation became PO was initiated by the introduction haemocyte surface receptor to the LPS, as a pathogenic molecule, that will activate some molecule responsible to the body defense system. Mechanism of fagocytosis activity on the injection of LPS to haemolymph of Red claw was intiated by the induction LGBP, thus body bind the pathogen such as bacteria and fungi and increase the coagulation process.

REFERENCES

Hernandez-Vergera, M.P., Grouse, D.B., Olvera-Novoa, M.A., & Davis, D.A. 2003, *Effets of Dietary Lipid Level and Source on Growth and Proximate Composition of Juvenile Redclaw (Cherax quadricarinatus) Reared under Semi-intensive Culture Condition*. Aquaculture 223: 107-115.

Hernandez-Lopez, J., Gollas-Galvan, T., & Vargas-Albores, F., 1996, Activation of the Prophenoloxidase System of the Brown Shrimp (Penaeus californiensis Holmes). Comparative of Biochamistry and Physiology, 113C: 61-66.

Hastuti, S.D., 2004, Disease and Immunology of Redclaw (Cherax quadricarinatus). A Thesis. The University of Queensland, Australia.

Jones, C.M., 1995, *Production of Juvenile Redclaw Crayfish, Cherax quadricarinatus (von Martens) (Decapoda, Parastacidae) II. Juvenile Nutrition and Habitat,* Aquaculture : 138. 239-245.

Jussila, J., 1997, Physiological Responses of astacid and Parastacid Crayfishes to Condition of Intesive Culture. Department of Applied Zoology and Veterinary Medicine University of Kuopio. Perth, Western Australia.

Lunde, H., & B. Robertsen, The Complement Component C3 is Produced by Hepatocytes and Behaves as an Acute-Phase Protein in Atlantic Salmon, Salmo salar L., after Injection 0f Beta 1,3-glucan. Developmental and Comparative Immunology, 1997. 21 :p.150.

Muzunic, L.A, Thomson, K.R., Morris, A;Webstera, C.D. Rouse, & D,B. Manomaitis, L., 2004, *Partial and Total Replacement of Fish Meal with Soybean meal and Brewer's Grains with yeast in Practical Diets for Australian Red Claw Crayfish Cherax quadricarinatus.* Aquaculture 230 : 359-376.

Selvin J. Lipton AP, 2004b, *Dendrilla nigre*, a Marine sponge, as Potential Source of Antibacterial Substances for Managing Shrimp Diseases. Aquaculture 236:277-283.