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Enhancement of Nonspecific Immune Response and Growth Performance of *Litopenaeus vannamei* by Oral Administration of Nucleotides

(PENINGKATAN RESPONS IMUN NONSPESIFIK DAN PERFORMA PERTUMBUHAN LITOPENAEUS VANNAMEI MELALUI PEMBERIAN NUKLEOTIDA SECARA ORAL)

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ABSTRACT

This research evaluated the nonspecific immune responseand growth of *Litopenaeus vannamei* fed nucleotide diet. In Laboratory, juveniles were reared in two groups of glass aquaria, each with three replications. Shrimps in group one were fed nucleotide diet and in group two were fedpellet four consecutive weeks. Total Haemocyte Count and Phenoleoxydase activity were evaluated at the end of feeding while growth was measured at two weeks interval. At the end of feeding, shrimps were intramuscularlyinjected with *Vibrio harveyi* $0.1x10^6$ cfu.shrimp^{-1}. In tambak, juveniles were raised in two groups of net cages (hapa), each with three replications. One group was fed nucleotide diet while the other wasfed pellet for four weeks. Total Haemocyte Count of shrimp fed nucleotide diet significantly increased up to 87% higher than shrimps fed pellet. Phenoleoxydase activity of shrimp fed nucleotides diet also increased isignificantly as compared to shrimp fed pellet (p=0.02). Higher resistance and growth were observed in shrimp fed nucleotide diet. In tambak, weight gain of shrimp fed nucleotide was 35.75% greater than shrimp fed pellet. Survival rate (83.24%) was higher than shrimp fed pellet (81.71%). As conclusion, oral administration of nucleotide at 400 mg.kg^{-1} diet could enhancethe nonspecific immune response, resistance, and growth of *L. vannamei*.

Keywords : Litopenaeus vannamei, nucleotides, total haemocyte count, phenoloxidase activity, resistance

ABSTRAK

Penelitian bertujuan untuk mengevaluasi respons imun nonspesifik, resistensi dan pertumbuhan udang vaname, *Litopenaeus vannamei*, yang diberi pakan nukleotida. Di laboratorium, udang juvenil dipelihara dalam dua kelompok akuarium kaca masing-masing dengan tiga ulangan. Udang pada kelompok pertama diberi pakan nukleotida dan udang pada kelompok kedua diberi pakan standar (pelet) selama empat minggu. *Total Haemocyte Count*dan Aktivitas Fenol-oksidase diukur pada akhir pemberian pakan perlakuan sedangkan pertumbuhan udang diukur setiap dua minggu. Pada akhir pemberian pakan perlakuan, udang disuntik secara intramuskuler dengan *Vibrio harveyi* $0{,}1x10^6$ cfu.udang^{-1}. Pada percobaan lapangan, juvenil ditangkap dari tambak dan dipindahkan ke dalam dua kelompok jaring (*hapa*) masing-masing dengan tiga ulangan. Udang dalam kelompok hapa pertama diberi pakan nukleotida sedangkan udang pada kelompok kedua diberi pakan pelet. Pakan perlakuan diberikan selama empat minggu. *Total Haemocyte Count* udang yang diberi pakan nukleotida meningkat secara nyata (p=0,01) mencapai 87% lebih tinggi dibandingkan dengan udang yang diberi pakan pelet. Aktivitas fenol-oksidase udang yang diberi pakan nukleotida juga meningkat secara nyata dinabdingkan dengan udang yang diberi pelet (p=0,02). Udang yang diberi pakan nukleotida memiliki resistensi dan pertumbuhan yang lebih tinggi (p<0,01). Di tambak, udang yang diberi pakan nukleotida memiliki berat akhir yang lebih besar dibandingkan dengan udang yang diberi pakan pelet. Perolehan berat udang yang diberi pakan nukleotida mencapai 35,75% lebih besar dari udang yang diberi pakan pelet. Kelangsungan hidup udang yang diberi pakan nukleotida (83.24%) lebih tinggi dibandingan dengan udang yang hanya diberi pakan pelet (81.71%). Sebagai simpulan, pemberian nukleotida secara oral sebanyak 400 mg.kg^{-1} pakan dapat meningkatkan respons imun nonspesifik, resistensi dan pertumbuhan udang vaname.

Kata-kata kunci : litopenaeus vannamei, nukleotida, total haemocyte count, aktivitas fenol-oksidase, resistensi

INTRODUCTION

Shrimp culturehas attracted serious attention from the government of Indonesia to be developed. However, since the last decade, many farmers and industries had suffered significant economic losses due to viral disease, not only in Indonesia but also in other countries (Moss *et al*., 2006). White Spot Syndrome Virus (WSSV) destroyed the industry since 1992/1993,

and since 2006, new disease namely infectious myonecrosis virus (IMNV) has been found to infect many shrimp aquaculture in Indonesia (Taukid and Nuraini, 2008). These two viral diseases are still unsolved.

Litopenaeus vannamei was first imported to Indonesia in 2000 to partially replace *Penaeus monodon* that was destroyed by WSSV (DKP, 2007). By the end of 2007, this species had been cultivated in more than 17 provinces in Indonesia. Infectious Myonecrosis Virus was first observed in Brazil in 2004 and now this virus has been found to infect shrimp culture in East Java, Bali, Nusa Tenggara and Sumatera (Taukhid and Nuraini, 2008). This disease develops gradually with a cumulative mortality 40-70% (Lightner, 2009).

A number of strategies that had been applied in diseases control included the use of probiotic bacteria, Specific Pathogen Resistance (SPR) or Specific Pathogen Free (SPF) shrimp, and biosecurity system. Many reports had shown that even though these methods can significantly increase production but disease still continues to occur because the susceptibility of shrimp to pathogen may differ according to life stages and the present of genetic mutation of pathogen in the environment. The use of antibiotic had gained serious concern due to negative effects such as accumulation of residue in shrimp tissues and environment and the appearance of drug-resistance pathogen. The use of immunostimulant is an alternative approach for disease control in shrimp aquaculture.

Immunostimulant is a substance that induces nonspecific immune response against infection of various pathogens simultaneously. This substance can be used as prophylactic treatment for unexpected disease or as suppressive treatment for latent and sub lethal pathogen (Nikl *et al*., 1993). Unlike vaccine, immunostimulant increases resistance of cultured shrimp against infectious pathogen simultaneously through stimulating the nonspecific immune response (Gannam and Schrok, 2001).

Immunostimulant can be grouped into bacteria and bacterial product, yeast, carbohydrate complex, nutrition factor, animal and plant extracts, and synthetic drugs (Sakai, 1999; Cook *et al*., 2003). Researches in fish and crustacean mostly used β-glucan because it occurs naturally, and no residue in fish and environment. The most common use of glucan products is *Saccaharomyces cerevisiae* (baker's yeast) and preparation of fungi *Schizophyllum commune* and *Selerotium glucanicum* (Sakai, 1999). Lopez *et al*., (2003) reported that administration of 2 g β-glucan per kg diet could induce immune response of *L. vannamei*. Chang *et al*. (2003) recommended the use of 2 g β-glucan per kg diet for 24 days for shrimp *P.monodon*, while Itami *et al*., (1998) recommended 2 g β-glucan per kg diet for *P. japonicus*.

This research used nucleotide as an immunostimulant in controlling shrimp diseases. Nucleotides are semi-essential nutrient that have essential physiological and biochemical functions including encoding and deciphering genetic information, mediating energy metabolism and cell signaling as well as serving as components of coenzymes, allosteric effectors, and cellular agonist (Li and Galtin, 2006). Application of nucleotide for disease control in aquaculture has obtained more attention since 2001. Publications concerning the application of nucleotide in fish showed that nucleotide could enhance immune response and resistance of fish against various pathogens simultaneously, increase growth and tolerance to stress. On the other hand, report on the use of nucleotide in shrimp was still unavailable or very limited. This present study was carried out to evaluate the nonspecific immune response, resistance and growth performance of *L. vannamei* fed nucleotides-supplemented diet.

RESEARCH METHODS

Shrimp. Shrimp juvenile was gathered from cultivation area at Bakauheni, South Lampung. Shrimps were placed into styrofoam boxes equipped with aerator and then transported to Fish Health Laboratory at the Bogor Institute of Agriculture, Bogor.

Nucleotides. Immunostimulant used in this research was pure nucleotides obtained from Sigma-Aldrich, United State of America. The nucleotides included adenosine monophosphate (AMP), guanosine monophosphate (GMP), uridine monophosphate (UMP), cytidine monophosphate (CMP) and inosine monophosphate (IMP).

Diet Preparation. Nucleotides with similar amount were mixed thoroughly. The mixture was then weighed to get the required amount, diluted into small amountof water, and mixed thoroughly into basal diet. The diet (pellet) was dried at room temperature, then coated with yellow egg and dried again at room temperature. Pellet was then put into plastic bags and stored in refrigerator until used.

Research Procedure and Data Collecting

Laboratory experiment. The research was conducted at the Fish Health Laboratory, Bogor Agriculture Institute. Shrimp juvenile was reared for two weeks in circular fiberglass tank for adaptation process. During acclimatization, the shrimps were fed basal diet three times a day at 09.00, 13.00, and 17.00 with a feeding rate of 3% bw.day^{-1}. Juveniles (mean 5.39±0.56 g)were then distributed into six glass aquaria

(60x30x30cm each) and divided into two groups of three. Each aquarium contained 50 L of water with 15 juveniles and was equipped with aerator and water recirculation. Juveniles at the first group were fed nucleotide-supplemented diet while juveniles at the second group were fed basal diet (pellet). Both groups were fed three times a day for four weeks at 3% bw.d^{-1}.During the experimental period, water quality was monitored daily to ensure good quality of water. Wastes and uneaten feed accumulated at the bottom of aquarium were removed by syphon and water exchange was done every three to four days depending on the condition of water.

Sample of haemolymph for measuring immune parameters was gathered at the end of the feeding period. Haemolymph was collected according to procedure suggested by Liu and Chen (2004). Shortly, about 1 mL of haemolymph was withdrawn from ventral sinus at the base of first abdomen using 1 mL syringe previously inserted with 0.1 mL anticoagulant. Anticoagulant used was EDTA (30 mM trisodium citrate, 0.34 M sodium chloride, 10 mM EDTA, pH 7.55, osmolality 780 $mOsm.kg^{-1}$).As much as 0.8 mL of anticoagulant was then added to the mixture to make the ratio between haemolymph and anticoagulant 1: 9

Immune Parameters. Immune parameters measured included total haemocyte count (THC) and phenoloxidase (PO) activity. To measure THC, about 50 µL of haemolymph was fixed in 10% neutral buffered formalin for 30 minutes. total haemocyte count was counted using haemacytometer under light microscope at 40x magnification. Phenoloxidase activity activity was measured based on dopachrome formation produced by L-DOPA. The measurement was done according to the procedure of Liuand Chen (2004). First, 1 mL of haemolymph-anticoagulant mixture was centrifuged at 700 g for 20 minutes at 4°C. Supernatant was removed and pellet was suspended into cacodylate-citrate buffer (0.01 M sodium cacodylate, 0.45 M sodium chloride, 0.10 M trisodium citrate, pH 7) and centrifuged again. Pellet was suspended into 200 µL cacodylate buffer (0.01 M sodium cacodylate, 0.45 M sodium chloride, 0.01 M calcium chloride, 0.26 M magnesium chloride, pH 7).

Aliquot of 100 µl was incubated with 50 µl trypsin (1 $mg.ml^{-1}$ cacodylate buffer) as activator for 10 minutes at 25-26°C. Then, 50 µL $_{L}$-DOPA ($_{L}$-3,4-dihydroxyphenylalanine, 3 $mg.mL^{-1}$ cacodylate buffer) was added, after five minutes, added 800 µL cacodylate buffer. Optical density (OD) 490 nm was measured using spectrophotometer.

Resistance. Four weeks after feeding, the shrimps were injected intramuscularly with 0.1 mL of *Vibrio harveyi* $1x10^{6}$ $cfu.mL^{-1}$ at the dorsal of third abdomen. During the challenged test, the shrimps were fed basal diet three times a day at 3% $bw.day^{-1}$. Mortality was observed every day for 14 days. Disease resistance was determined based on survival rate of shrimp after challenge test. Where : SR (%) = Nt/No x 100; SR = survival rate; Nt = number of live shrimp at time *t;* No = number of live shrimp at the beginning of experiment

Growth . Growth of shrimp were measured every two weeks namely at day 14^{th} and 28^{th} . shrimp growth was calculated based on the formula as follows, where : G=Wt–Wo; G= growth; Wt = final weight of shrimp (g); Wo = initial weight (g)

Field Experiment. The experiment was conducted at the shrimp culture area of Bakauheni, South Lampung. Juveniles (mean weight 4.5 g) were caught from pond using a cast net and moved into six *hapa* (net cages, measuring 2x1x1m each) situated at the same pond at the density of 175 juveniles.$hapa^{-1}$. These six *hapa* were divided into two groups, each with three replications. The distance between group one and two was 2 m while the distance of bottom part of *hapa* from pond bottom varied from 10 to 30 cm depending on the slope of pond bottom. The depth of *hapa* sank under the water surface was 75 cm.

Shrimps in group one of *hapa* were fed pellet supplemented with 400 mg nucleotides/kg of pellet for four weeks at 4% of body weight per day, three times a day (09.00, 13.00 and 17.00). Shrimps in group two were fed pellet without supplementation of nucleotides. Feeding was done by placing the pellet in an *anco* and the anco was sank slowly to the bottom of *hapa.*

Growth Performance and Resistance. Growth performance of shrimps was evaluated through absolute growth and average daily gain while shrimp resistance was established based on survival rate achieved at the end of trial. Weighing of shrimp body weight was done at two weeks interval. Where: ADG = (ABW_{t} – ABWo / T; ADG = Average Daily Gain (g); ABWt= average body weight at the end of trial (g); ABWo = initial average body weight (g); T=duration of experiment (day)

Data Analysis. Data were presented as mean±Sd. The effect of immunostimulants on THC, PO activity, resistance and growth of shrimp was evaluated through analysis of variance

RESULTS AND DISCUSSION

Laboratory Experiment

Total haemocyte count. Supplementation of nucleotide in shrimp pellet enhanced total haemocyte count (THC) of shrimp. One way ANOVA demonstrated that THC of shrimp fed nucleotide-supplemented diet was significantly different from that of shrimp fed pellet without

Table 1. Total haemocyte count, phenoloxidase activity and surviral rate of *L. vannamei* fed nucleotides diet for four weeks

Diets	THC(x10^7 cells/mL)	PO Activity	Survival Rate (%)
Pellet	1.119 ± 0.26^a	0.304 ± 0.028^a	45.83 ± 7.22^a
*Nucleotides*Sig.	2.090 ± 0.437^b p=0.01	0.632 ± 0.162^b p=0.02	79.17 ± 7.22^b P<0.01

Mean value with different superscript was significantly different

supplementation of nucleotides (p=0.01). After four weeks of feeding, THC of shrimpfed nucleotides diet increased up to 2.090 ± 0.437 cells.mL^{-1} or 87% higher than THC of shrimp fed pellet (Table 1).

Similar result was observed in the previous research in which THC of shrimp fed nucleotide diet at 400 mg.kg^{-1} pellet for four weeks increased up to 76% higher than control (Manoppo *et al*., 2009). In juveniles of *P. monodon*, Hill *et al.* (2006) found that shrimps fed nucleotides diet (Vannagen 0.2%) had THC 100% higher than shrimp fed diet without supplementation of nucleotides while on larger shrimp, the number of THCwas 30% higher than control shrimp. Nucleotide is a semi essential nutrient required for growth and cell replication (Barnes, 2006). Thus, supplementation of nucleotide in shrimp diet may optimize proliferation of cells including immune cells (Sajeevan *et al*., 2006). In addition, nucleotides supplemented to shrimp pellet will bind to molecule receptors present at the surface of phagocyte cells (Raa, 2000). The cells then become more active for phagocytosis of pathogen or foreign particles and at the same time, they produce signal molecules (cytokine) that stimulate the production of new haemocyte.

Phenoloxidase activity. Oral administration of nucleotide positively enhanced PO activity (Table 1). Analysis of variance showed that PO activity of shrimp fed nucleotide-diet for four weeks was different from that of shrimp fed pellet without supplementation of nucleotides (p=0.02). The process of how nucleotide increases PO activity was still unclear, but Li and Galtin (2006) assumed that nucleotide added to the diet will participate in cell signaling pathway as well as be used as nutrient for biosynthesis processes. Several reports had shown that immunostimulant such as β–glucan could enhance PO activity of *P. monodon*(Chang *et al*., 2003), *L.vannamei* (Lopez *et al.,*2003), and *Macrobrachium rosenbergii* (Sahoo *et al*., 2008). β–glucan enhanced PO activity after binding to beta glucan binding protein(Li *et al.,* 2008; Vargas-Albores and Yepiz-Plascencia, 2000). Once it binds, inactive proenzyme PO (proPO) is activated to be PO enzyme necessary for melanization. Lopez *et al.* (2003) reported that β-glucan added to the diet will induce cell activating factors in haemocyte, thus increase PO activity and phagocytosis. The same process may occur on shrimp fed pellet supplemented with nucleotides.

It was found in this research that the higher the THC the higher the PO activity. This is due to PO present in haemoluymph in form of inactive proenzyme called proPO (Vargas-Albores and Yepiz-Plascencia, 2000). The proPO is produced and released into haemolymph by haemocytes, mainly granular and semi granular cells. In normal condition, the higher the total haemocytes the higher the production and the release of proPO, thus PO activity will be higher too.Phenoloxydase activity of shrimp fed nucleotides diet was categorized as high activity (>0.35) while shrimp fed pellet without supplementation of nucleotides was normal, that was between 0.2 – 0.35 (Gullian *et al*., 2004).

Resistance. Disease resistance was determined based on survival rate of shrimp after challenged with *V. harheyi*1x10^6 cfu. $shrimp^{-1}$. Mortality occurred one day after challenge, and continued until four days post-challenge. Afterward, no mortality was observed in both treatments. Supplementation of immunostimulant nucleotides positively affected the resistance of shrimp. One way Anova showed that at 28 days post-challenge, survival rate of shrimp fed nucleotides diet was significantly different (p=0.003) from shrimp fed pellet (Table 1).

There was no report concerning the effect of supplementation of nucleotide on shrimp resistance to disease. In fish, Li *et al*. (2004) reported that production of oxidative radical of blood neutrophil of hybrid striped bass increased after fed nucleotide diet for six to seven weeks and infected with *Streptococcus iniae,*and survival of fish (80%) was higher than that of fish fed free nucleotide diet (60%). Burrels *et al.,* (2001) also reported that mortality of rainbow trout fed nucleotide diet (Optimun) for two weeks and challenged with Infectious Salmon Anaemia Virus(ISAV)) was 35.7% while mortality of fish fed basal diet was 48%. Sakai *et al.,* (2001) reported that application of nucleotide isolated from yeast RNA at 15 mg.$fish^{-1}$ for three days on *Cyprinus carpio* 100 g increased resistance of fish to *A. hydrophila* infection. Phagocytosis activity, respiratory burst, complement serum and lysozyme activity in fish fed nucleotides diet increased. Burgents *et al*., (2004) found the increase of *L. vannamei* resistance to Vibrio if the shrimp fed diet containing *S. cerevisiae.* In this research, it was found that supplementation

Table*2*.Growth of *L. vannamei*after feeding nucleotides diet for four weeks

Diet	Initial Weight (g)	Final Weight (g)		Weight Gain (g)	
		14thday	28th day	14th day	28th day
Pellet	5.39±0.56	7.37±0.36	8.25±0.71	1.98±0.36^{a}	2.86±0.71^{a}
Nucleotides	5.39±0.56	7.71±0.81	10.12±0.57	2.32±0.81^{a}	4.73±0.57^{b}

Mean value with different superscript was significantly different ($p<0.01$)

Table 3. Growth performance of *L. vannamei* raised in hapa and fed nucleotides diet 400 mg/kg pellet for four weeks

Diet	Wo (g)	Wt (g)	G (g)	ADG (g)
Pellet:				
Week-2 (n=24)	4.50	7.4±0.17	2.54±0.13^{a}	0,180±0.059
Week-4 (n=45)	4.50	10.01±1.36	5.51±1.36^{a}	0.204±0.049
Nucleotides:				
Week-2 (n=30)	4.50	7.92±0.91	3.42±0.91^{b}	0.243±0.066
Week-4 (n=45)	4.50	11.98±1.08	7.48±1.08^{b}	0.277±0.039
Tambak:				
Week-2 (n=30)	4.50	6.57±0.40	2.07±0.40	0.147±0.028
Week-4 (n=30)	4.50	8.93±0.21	4.43±0.21	0.163±0.007

Mean value with different superscript was significantly different ($p<0.01$)
Wo : mean initial weight (g); Wt : mean final weight at t (g);
G : Weight Gain (Wt-Wo); ADG: average daily gain (g)

Table 4.Survival rate and feed efficiency of *L. vannamei* fed nucleotides diet for four weeks

Diet	N_o	Wo	N_t	W_t	W_G	SR (%)	Diet amount (g)	FCR
Nucleotides:								
Hapa 1	175	787.5	164	1972.92	1185.42	93.71	1290	1.09
Hapa 2	175	787.5	141	1677.90	890.40	80.57	1290	1.45
Hapa 3	175	787.5	132	1584.00	796.50	75.43	1290	1.62
Average		787.5	146	1744.94	957.44	83.24±9.2	1290	1.35
Pellet:								
Hapa 4	175	787.5	138	1439.34	651.84	78.86	1290	1/98
Hapa 5	175	787.5	150	1420.50	633.00	85.71	1290	2.04
Hapa 6	175	787.5	141	1428.33	640.83	80.57	1290	2.01
Average		787.5	143	1429.39	641.89	81.71±3.56	1290	2.01

No : initial number of shrimp; Nt : number of shrimp at the end of experiment;
Wo: initial weight of shrimp (g); Wt: final weight of shrimp (g);
W_G: weight Gain (g) = Wt – Wo; SR: survival rate (%) = Nt/No x 100;
FCR (food conversion ratio) =food consumed (g)/weight gain(g)

of nucleotides into shrimp pellet could induce resistance of *L. vannamei* to pathogen and potential to be applied in management of shrimp diseases.

Growth. Oral administration of nucleotides for two weeks did not induce growth of shrimp ($p>0.05$), but after feeding for four weeks, growth of shrimp fed nucleotide died was significantly higher ($p<0.01$) than shrimp fed pellet without nucleotides supplementation (Table2). Weight gain of shrimp fed nucleotide diet was 4.73 g or 65.38% greater than that of shrimp fed pellet. Similar result was observed in the previous research where weight gain of shrimp fed nucleotide diet at 400 mg.kg^{-1} pellet for four weeks achieved 50.74% greater than control shrimp (Manoppo *et al*., 2009). In salmon, Burrells *et al*., (2001) found that the growth of fish increased after eight weeks fed with nucleotides diet (Optimun) at a dose of 2 g.kg^{-1} diet. Nucleotides diet also induced growth and immune response of grouper *Epinephelus malabaricus*(Lin *et al,*. 2009).

In normal condition, *de novo* synthesize of nucleotide is enough to support growth (Li and Galtin, 2006). Research reports showed that nucleotide supplemented in feed could promote growth of fish and crustacean at the early stage to fulfill the need of production and cell replication. Nucleotide had also been long used in nutrition mainly as chemo-attractant for fish and animals. Adenosine and inosine are good

chemo-attractant widely used for fish and crustacean. These substances increase feed intake, and therefor reduce leaching of nutrients into the water (Li *et al*., 2007). Thus the increase growth of shrimp fed nucleotides diet might be resulted from the increase of feed efficiency and food intake.

Field Experiment

Growth performance. After feeding for two weeks, growth of shrimp fed nucleotides-supplemented diet was significantly different as compared to shrimp fed pellet only ($p<0.01$). This significantly different continued till the end of the trial. After two weeks of feeding, final weight of shrimp fed nucleotides diet was 7.92±0.91g (weight gain 3.42±0.91g) or 34.65% heavier than shrimp fed pellet or 65.22% heavier than shrimp raised in brackish water pond (Table 3). After four weeks of feeding, mean weight of shrimp fed nucleotides-supplemented diet achieved 11.98±1.08 g and mean weight gain was 7.48±1.08 g or 35.75% greater than weight gain of shrimp fed nucleotides-free diet.Average daily gain of shrimp fed nucleotides-supplemented diet was 0.277±0.039 g. It was found in this research that food conversion ratio (FCR) of shrimp fed nucleotides diet was 1.35 while shrimp fed pellet was 2.01 (Table 4). According to Lightner (2009), normal food conversion ratio of *L. vannamei* was 1.5. Thus, oral administration of nucleotides for four consecutive weeks could promote shrimp growth.

Reports on the use of nucleotides in shrimp culture were still limited. In the previous laboratory experiment, administration of 400 mg nucleotides.kg^{-1} pellet displayed significant effect on growth after feeding for four weeks. Shrimp with mean weight of 6.0±0.5g achieved 11.05±0.40g after four weeks. Other laboratory report (Li *et al*.,2007) showed that shrimp (mean weight 0.84g.ind^{-1}) grew up to 10.96 g after five weeks fed with nucleotides diet at 400 mg.kg^{-1} diet. In this field experiment, application of nucleotides with similar dose could promote significant growth after two weeks of feeding and continued until four weeks of feeding.

Resistance. Survival rate of shrimp in both treatments was high and there was no significant different ($p>0,05$) between the two treatments (Table 4). Similar result was observed in our previous laboratory research where after challenge with *V. harveyi*, resistance of shrimp fed nucleotides diet was 83.33% (Manoppo *et al.,* 2009). In this field experiment, survival rate of shrimp fed nucleotides diet was 83.24% while shrimp fed pellet was 81.71%. Shrimp mortality occurred mostly due to cannibalism and the present of infectious myonecrosis virus (IMNV). This virus was first detected in Indonesia in 2006 and still untreated until now.

Infectious myonecrosis (IMN) is a viral disease of penaeid shrimp caused by infection with infectious myonecrosis virus(Lightner, 2009). The principal host species in which IMNV is known to cause significant disease outbreaks and mortalities is *L. vannamei*. Outbreaks of IMN with sudden high mortalities may follow stressful events such as capture by cast-net, feeding, sudden changes in salinity or temperature, etc., in early juvenile, juvenile, or adult *L. vannamei* in regions where IMNV is enzootic, or in *L. vannamei* introduced from infected regions or countries. Such severely affected shrimp may have been feeding just before the onset of stress and may have a full gut, and shrimp in the acute phase of IMN disease will present focal to extensive white necrotic areas in striated (skeletal) muscles, especially in the distal abdominal segments and tail fan, which can become necrotic and reddened in some individual shrimp. Severely affected shrimp become moribund and mortalities can be instantaneously high and continue for several days. Mortalities from IMN range from 40 to 70% in cultivated *P. vannamei*, and food conversion ratios of infected populations increase from normal values of 1.5 to 4.0 or higher.

CONCLUSION

Oral administration of nucleotides for four weeks could enhance nonspecific immune response, resistance and growth performance of *L. vannamei*

SUGGESTION

Exploration of proper nucleotides sources, easily available and inexpensive is required for future application of nucleotides in shrimp health management practices.

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