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EFFECTS OF FEED SUPPLEMENTATION OF *Nodulisporium* sp. KT29 INDUCED BY *Vibrio harveyi* CELLS ON PRODUCTION PERFORMANCE OF PACIFIC WHITE SHRIMP *Litopenaeus vannamei* CULTURED UNDER MARINE CULTURE SYSTEM

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ABSTRACT

This current study aimed to investigate the effects of *Vibrio harveyi* induced *Nodulisporium* sp. KT29 administration on enhancement of bioactive compound concentration, surface of intestinal structure, and production performance of Pacific white shrimp (*Litopenaeus vannamei*) cultured in the sea. The post larvae (PL) 10 of *L. vannamei* was cultured at density of 1000 shrimp/m^3 for 30 days in the sea. Completely randomized design was arranged, consisting of 3 treatments with three replications, i.e C (control 0 mL/kg), NP (*Nodulisporium* sp. KT29 without induction of *V. harveyi* 20 mL/kg), NK (*Nodulisporium* sp. KT29 with induction of killed *Vibrio harveyi* 20 mL/kg), NL (*Nodulisporium* sp. KT29 without induction of living *V. harveyi* 20 mL/kg). The results revealed that NK treatment showed the best effects on rising concentration of bioactive compounds, production performance, and improving the density of shrimp intestinal structure compared to control.

Key words: *Nodulisporium* sp. KT29, bacterial induction, β-glucan, *Litopenaeus vannamei*, production performance, *Vibrio harveyi*

INTRODUCTION

The Indonesian sea has an enormous potential for aquaculture practices, but it has not reached the optimum use. In 2014, marine culture sector accounted for 19.94% to the Indonesian fishery production (DSIC MMAF RI, 2015). Based on this condition, development of marine shrimp farming is regarded as a promising approach to enhance aquaculture production. The culture of Pacific white shrimp using floating net cages set in the sea has several advantages compared to that in brackish-water ponds; it does not produce accumulation of organic waste from debris and does not require energy for water replacement and aeration (Zarain-Herzberg *et al.,* 2010). However, marine shrimp farming practice is also associated with several disadvantages such as inconsistent production performance which is not comparable with shrimp culture in brackish-water ponds (Effendi *et al.,* 2016), dynamic natures of marine environments and the existence of predators that may lead to physiological stress (Beveridge, 1984; Swann *et al.,* 1994). Stress on the shrimp will lead to the increasing of the energy use for the body's defense system that will decrease the production performance (Peterson and Walker, 2002).

The application of materials with immunestimulant and eco-friendly properties has been used in improvement of immune system, including synbiotic (Djauhari *et al.,* 2017) and natural materials (Babu *et al.,* 2013). *Nodulisporium* sp. KT29 is a natural endophytic fungus isolated from red alga

Euchema edule supplied from Takalar, South Sulawesi. The fungus is reported to contain immunestimulant compounds, antioxidants such as β-glucan, saponin, polyphenol, and phytosterol (Tarman *et al.,* 2011), capable of increasing production (Saputra *et al.,* 2016) and shrimp immune system (Wahjuningrum *et al.,* 2016). However, administration of *Nodulisporium* sp. KT29 metabolite at high dose may lead to higher production cost, thus a study on determination of effective-cost formula is required.

The improvement of bioactive compound level in fungus can be carried out through some methods such as bacterial induction, salinity, pressure, light, and temperature (Tarman *et al.,* 2011). Cueto *et al.,* (2001) found that bacterial induction together cultured with *Pestalotia* sp. could increase concentration of antibiotic compounds. In addition, addition of carbohydrate sources such as glucose, fructose, sucrose, and starch enabled to enhance secondary metabolite of *Pleurotus ostreatus* (Rana and Dahot 2017). This present work employed bacterial method in increasing bioactive compounds of *Nodulisporium* sp. KT29 for production of secondary metabolite. Pathogenic bacteria, *Vibrio harveyi*, were found in both fresh water and sea (Widanarni *et al.,* 2012; Wahjuningrum *et al.,* 2016), and reported to contain lip-

opolysaccharide (Pandey *et al.,* 2016). Mearns-Spragg *et al.,* (1998) reported that administration of pathogenic bacteria such as *Escherisia coli* together cultured with alga *Fucus vesiculosus* could produce secondary metabolites such as saponin. This study was to understand the effects of supplementation of *Nodulisporirum* sp. KT29 induced by *V. harveyi* on improvement of bioactive compound production, surface of intestinal structure, and production performance of *L. vannamei* under marine shrimp farming practice.

MATERIALS AND METHODS

Preparation of *Nodulisporium* sp. KT29 metabolite: Fungal isolate *Nodulisporium* sp. KT29, was obtained from Aquatic Product Microbiology Laboratory, Department of Aquatic Product Technology, Bogor Agricultural University, Indonesia. *Nodulisporium* sp. KT29 was cultivated according to a modified method of Tarman *et al.* (2011). Rejuvenation of *Nodulisporium* sp. KT29 isolate was carried out on potato dextrose agar (PDA) for 7 days, then cut in the shape of a cube for preculture in 100 mL of potato dextrose broth (PDB) for 7 days at 28-30°C in static condition. The suspension obtained from the pre-culture process was treated by *V. harveyi* induction treatments. Prior to induction process, *V. harveyi* suspension was prepared by growing *V. harveyi* cells in 300 mL SWC (sea water complete) broth and incubited in a thermoshaker at 28-30°C, 160 rpm for 18 hours. The content of *V. harveyi* in the suspension was approximately 10^8 CFU mL^{-1}.

The induction treatments included induction of killed *V. harveyi* cells (NK), the induction of living *V. harveyi* cells (NL), and without any induction (NP). For NK treatment, *V. harveyi* cells were killed through sterilization using autoclave (121°C). The *Nodulisporium* sp. KT29 suspension obtained from the pre-culture process was further transferred into 250 mL of PDB and mixed with 5% (12.5mL) suspension of killed *V. harveyi* (NK), 5% (12.5 mL) suspension of living *V. harveyi* (NL) and without addition of *V. harveyi* suspension (NP), to produce fungal biomass. *V. harveyi* (NP) and *Nodulisporium* sp. KT29-*V. harveyi* (NK and NL) suspensions were incubated with thermoshaker at 28-30°C and 120 rpm. After 14 days of incubation, all suspensions (NK, NL, and NP) were filtrated using Whatmann paper (mesh size 0.45 µm), then the filtrate were evaporated (40°C, rotary evaporator) for 1 hour to remove the water. The evaporated results (metabolites) were used for *in vivo* test. To analyze the content of bioactive compounds of *Nodulisporium* sp. KT29, each harvested metabolite was extracted through three-tiered maceration with a ratio *Nodulisporium* sp. KT29 and EtOAc of 1:2 (100 mL *Nodulisporium* sp. KT29:200 mL EtOAc) for 24 hours (28-30 °C; 120 rpm).

***In vitro* test:** *Nodulisporium* sp. KT29 metabolites treated by *V. harveyi* treatments (NP, NK, and NL) were analyzed their bioactive compounds content including β-glucan and phytochemical compounds. The analysis of β-glucan levels was performed using a spectrophotometer (Stecher *et al.,* 2004). Phytochemical compounds contained in *Nodulisporium* sp. KT29 metabolites consisting of saponin, polyphenol and phytosterol were analyzed their levels using HPLC (high performance liquid chromatography) (Rahar *et al.,* 2017).

Preparation of experimental feed: This study used the commercial feed (Fengli MS; PT. Matahari Sakti, Indonesia) containing 40% protein, 5% fat, 2% fiber, 13% ash, and 11% moisture. The commercial feed was milled and was added 0.1% vitamin C (1g/kg feed) and 3% CMC (carboxyl methyl cellulose) (30 g/kg feed) as the control feed (C), while the feed for treatments was also supplemented with *Nodulisporium* sp. KT29 metabolites treated by *V. harveyi* induction treatments (NP, NK and NL) at a dose of 20mL/kg. Each mixture was re-pelleted, then mashed into crumbles.

***In vivo* test:** The *in vivo* test was performed through a completely randomized design (CRD) with four treatments and three replications. Treatments of *in vivo* test included feed without the supplementation of *Nodulisporium* sp. KT29 as control (K), feed with the supplementation of *Nodulisporium* sp. KT29 without the induction of *V. harveyi* cells (NP), feed with the supplementation of *Nodulisporium* sp. KT29 induced by killed *V. harveyi* cells (NK), and feed with the supplementation of *Nodulisporium* sp. KT29 induced by living *V. harveyi* cells (NL). The rearing of Pacific white shrimp was conducted in floating net cages, Sea Farming Center, Center for Coastal and Marine Resources Studies (CCMRS), Bogor Agricultural University, Semak Daun Island, Kepulauan Seribu, Indonesia. Pacific white shrimps used were PL-10 shrimps (weight 0.014 ± 0.01 g, length 0.8 ± 0.01 cm) derived from the hatchery of PT. Suri Tani Pemuka, Anyer, Banten, Indonesia. The shrimps were stocked into floating net cages with a stocking density of 1000 individuals/m^3. The shrimps were reared for 30 days and were fed four times a day (06:00 am, 10:00 am, 02:00 pm, and 06:00 pm) at satiation level. During *in vivo* test, the measurements of water quality parameters were performed and the parameters ranges were maintained at the optimum ranges for the life of Pacific white shrimp, i.e

temperature at 26-30°C, pH at 7.6-8.56, dissolved oxygen level at 5.2-8.5 mg/L, salinity at 34-36 ppt, alkalinity at 94-243 mg/L, total ammonia nitrogen at 0.07-0.8 mg/L, nitrite at 0.01-0.04 mg/L, and nitrate at 0.19-0.39 mg/L.

Experimental parameters: The research parameters included bioactive content of *Nodulisporium* sp. KT29, intestinal surface structure, and production performance of the shrimp including survival rate (SR), specific growth rate (SGR), long growth (LG), weight gain (WG), feed conversion ratio (FCR). The parameters were observed after 30 days of in vivo experiment in the sea. Surface of shrimp intestinal structure was observed under Scanning Electron Microscope, SEM (JSM-5310 LV, Japan) according to method of Goldstein *et al.*, (1992).

Statistical analysis: Data of SR, SGR, LG, WG, and FCR were evaluated using Analysis of Variance (ANOVA) in SPSS 16. Significant differences between means were compared using Duncan test at $P<0.05$. Descriptive analysis (tables and figures) was carried out to evaluate the content of bioactive compounds of *Nodulisporium* sp. KT29 treated by *V. harveyi* induction treatments and the intestinal surface structure of Pacific white shrimp after *in vivo* experiment.

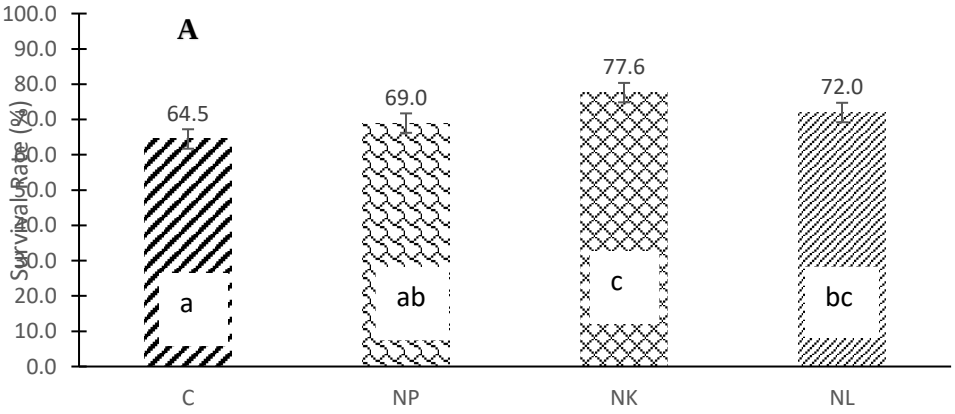
RESULTS

The content of bioactive compounds of *Nodulisporium* sp. KT29 induced by *Vibrio harveyi*: Results of the analysis of the content of bioactive compounds of *Nodulisporium* sp. KT29 treated by *V. harveyi* induction treatments demonstrated that NK showed the highest levels of bioactive compounds (1.34% β-glucan, 48 ppm saponin, 58 ppm polyphenol, and 148 ppm phytosterol). Those values were followed by values on NL and NP, respectively (Table 1).

Table 1: Bioactive compound content of *Nodulisporium* sp. KT29 without induction treatment (NP), induced by killed (NK) and living (NL) *Vibrio harveyi* cells

Bioactive compounds	Treatments		
	NP	NK	NL
β-glucan (%)	0.66	1.34	0.95
Saponin (ppm)	25	48	39
Polyphenol (ppm)	35	58	49
Phytosterol (ppm)	125	148	139

Production performance of Pacific white shrimp cultured under marine culture system: The supplementation of *Nodulisporium* sp. KT29 induced by killed *V. harveyi* (NK) at dose of 20 mL/kg for 30 days significantly affected production performance of Pacific white shrimp ($P<0.05$) in comparison with control (C). Furthermore, NK treatment resulted in the best production performance, including survival rate (SR) of 77.0 ± 4.16% (Figure 1A), specific growth rate (SGR) of 11.4 ± 0.1 % / day (Figure 1B), feed conversion ratio (FCR) of 1.8 ± 0.24 (Figure 1C), long growth (LG) of 4.8 ± 0.08 cm (Figure 1D), and weight gain (WG) of 0.46 ± 0.016 (Figure 1E).



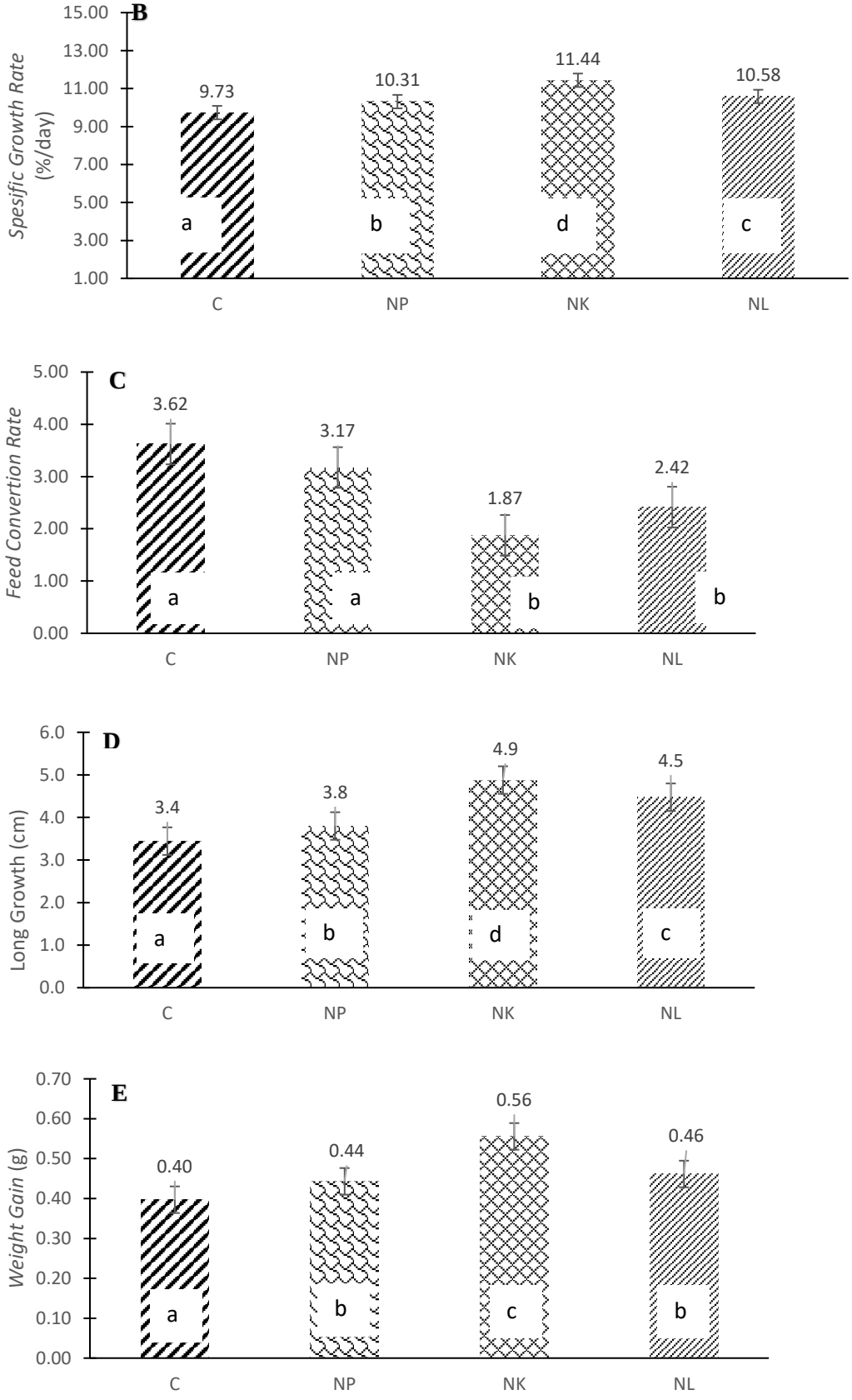


Figure 1. Production performance of *L. vannamei* under marine culture system: Survival Rate (A), Specific Growth Rate (B), Feed Conversion Ratio (C), Long Growth (D), Weight Gain (E). Treatments included Control (K), *Nodulisporium* sp. KT29 without induction of *V. harveyi* (NP), with induction of killed *V. harveyi* (NK), and with induction of living *V. harveyi* (NL). Different superscripts showed significant differences at $P<0.05$.

Intestinal surface structure of Pacific white shrimp: The NK and NL treatment showed a positive effect on the intestinal surface structure of Pacific white shrimp. Pacific white prawns on NK and NL treatment have a more dense and compact intestinal surface structure compared to treatment C and NP (Figure 2).

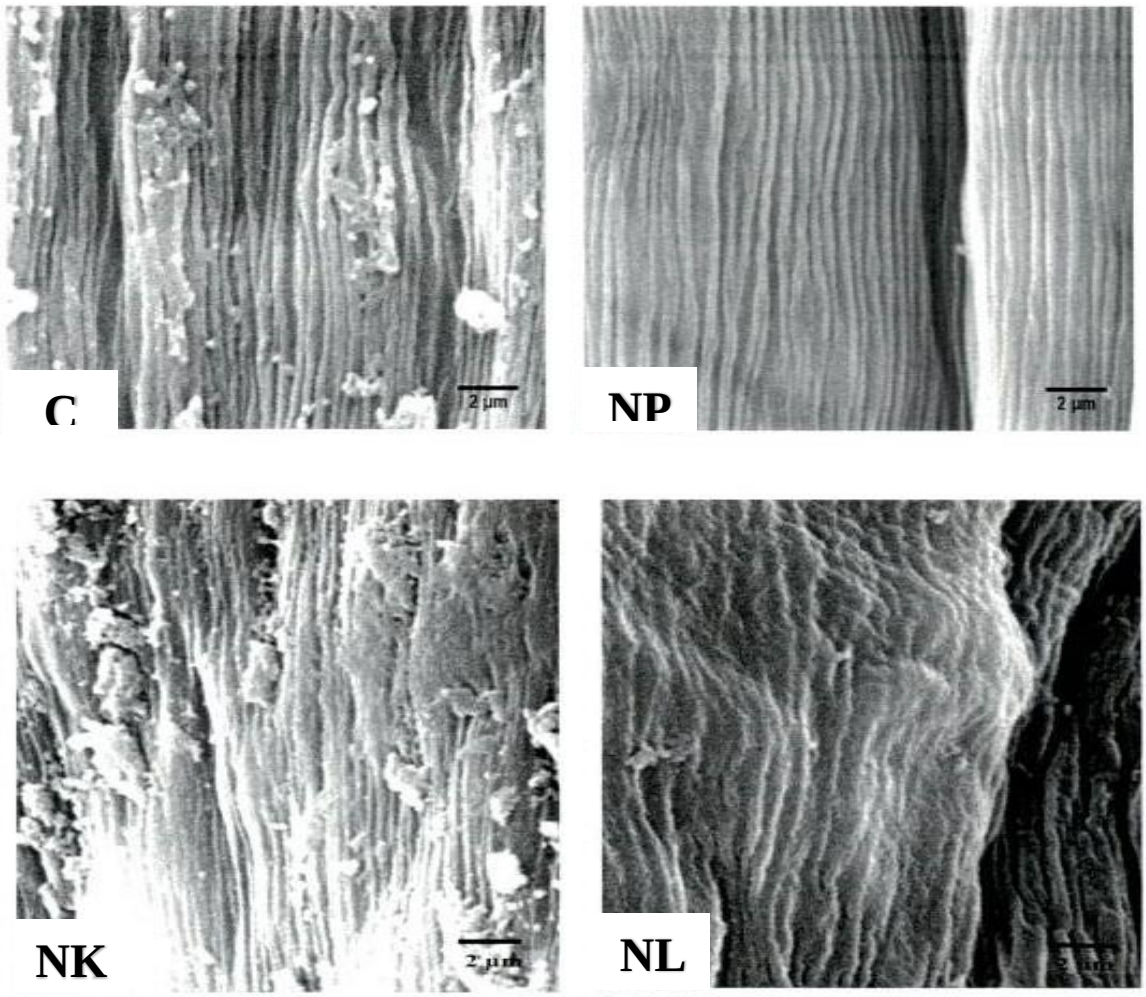


Figure- 2: The intestinal surface structure of *L. vannamei* without supplementation of *Nodulisporium* sp. KT29 (C), supplemented with metabolite of *Nodulisporium* sp. KT29 without induction treatment (NP), with induction of killed (NK) and living (NL) *V. harveyi* cells for 30 days of experiment under marine culture system

DISCUSSION

Nodulisporium sp. KT29 induced by killed *V. harveyi* (NK) and living *V. harveyi* (NL) prior to massive culture could produce higher secondary metabolite (Table 1). In NK treatment, presence of foreign component, *V. harveyi,* was able to induce formation of defence system in *Nodulisporium* sp. KT29 against the foreign component. The bacteria were then used as source of nutrition to produce secondary metabolite. Meanwhile, NL treatment promoted competition for habitat and nutrition, thus enhancing activity of *Nodulisporium* sp. KT29 to deactivate *V. harveyi* and use them as nutrition sources for production of secondary metabolite. These findings suggest that bacterial induction, recognized as foreign component, enables to increase concentration of secondary metabolite produced by fungus as a defence action (Calvo *et al.,* 2002; Ipcho *et al.,* 2016). The increasing of bioactive compounds promoted by induction of fungus was in accordance with Tarman *et al.,* (2011), finding that fungi *Mycelium sterilium* induced by 5% of killed *Staphylococcus aureus* at initial culture could increase concentration of bioactive compounds.

Bioactive compounds had positive effects on the growth performance and the immunity of Pacific white shrimp cultured under marine culture system, because those bioactive compounds had potentials as immunostimulant, antibacterial, antiviral, antioxidant, and anti-stress (Citarasu, 2010). The β-glucan and phytosterol are immune-

modulators that can enhance the immunity of organisms (Greiner *et al.*, 2001; Chen *et al.*, 2016), thus Pacific white shrimp was able to deal with physiological stress due to environmental changes. Polyphenol acts as antioxidant protecting cells from free radicals that cause oxidative stress (Mattson and Cheng, 2006). Saponin is an antibacterial property protecting an organism from a pathogenic infection (Su *et al.*, 2008).

The administration supplementation of *Nodulisporium* sp. KT29 in feed for 30 days of experiment enabled to raise production performance of *L. vannamei* under marine culture system, especially promoted by NK treatment at dose of 20mL/kg (Figure 1). Secondary metabolite produced by *Nodulisporium* sp. KT29 plays role in enhancing immune system and growth performance of *L. vannamei*, thus improving capability of utilizing nutrition allocated for shrimp growth (Bai *et al.*, 2014; Ekasari *et al.*, 2016). The plausible mechanism of the bioactive compounds included absorption of β-glucan in digestive tract and neutralized in macrophage to form smaller size, thus absorbed by microvilli. The absorbed nutrition was allocated for improving the growth (Dimitroglu *et al.* 2010; Bai *et al.* 2014).

Supplementation of *Nodulisporium* sp. KT-29 exhibited a remarkable change in the intestinal surface structure of *L. vannamei*, indicated by enhanced density of microvilli (Figure 2). The best result was attributed to NK and NL treatments, yielding the improved microvilli surface which affected nutritional absorption and feed efficiency. Dimitroglou *et al.*, (2010) found that administration of MOS as immunostimulant improved the density and length of the microvilli in the anterior and posterior of intestine, leading to enhanced nutritional absorption in *Sparus aurata*. Supplementation of *Nodulisporium* sp. KT29 induced by *V. harveyi* also positively affected FCR of the shrimp. This may indicate that presence of phytosterol and saponin in the secondary metabolite of *Nodulisporium* sp. KT29 promotes enhanced feed efficiency. Couto *et al.*, (2014) rep-orted that administration of phytosterol and saponin, both in single and blended form, resulted in better feed utilization by gilthead fish (*Sparus aurata*).

CONCLUSION

Nodulisporium sp. KT29 induced by killed *V. harveyi* (NK) could raise production of secondary metabolite. Additionally, NK treatment also resulted in the best production performance, and improved density of intestinal surface structure of *L. vannamei* under marine culture system.

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