Original article DOI: 10.19027/jai.21.1.41-51

Functional effects of natural silicate, yeast and saponins based product on the growth and health status of the Pacific white shrimp *Litopenaeus vannamei*

Efek fungsional bahan berbasiskan silikat alamiah, ragi dan saponins terhadap pertumbuhan dan status kesehatan udang putih *Litopenaeus vannamei*

Romi Novriadi[1]*, Djumbuh Rukmono[1], Benny Shapira[2], Arik Farzeli[2]

[1] Department of Aquaculture, Jakarta Technical University of Fisheries, Agency for Marine and Fisheries Research and Human Resources, Ministry of Marine Affairs and Fisheries, Jl. AUP, Pasar Minggu, South Jakarta, Jakarta, Indonesia,

[2] Research & Development, Phibro Animal Health Corporation, Teaneck, NJ 07666, USA

*Corresponding author: novriadiromi@yahoo.com

ABSTRACT

In this study, a commercial product of natural silicates and yeast (NSY) and saponins from *Yucca schidigera* and *Quillaja saponaria* plants (SBP) were used to evaluate their nutritional effects on growth and health status of pacific white shrimp *Litopenaeus vannamei*. Four isonitrogenous and iso-lipidic experimental diets were formulated to contain 0%, 0.3% NSY, 0.2% SBP and 0.6% NSY. After 60 days, shrimp were sampled and standard haemato-immunological parameters were measured. The growth performances of shrimp were significantly affected by the dietary inclusion of NSY and SBP, whereas the inclusion of NSY and SBP provide a better growth compared to the control group. Additionally, the inclusion of NSY and SBP significantly enhance the total haemocytes count and lysozyme activity in shrimp compared to control. Therefore, NSY and SBP can be regarded as the functional ingredients in shrimp diet to improve the growth and non-specific immune function of shrimp.

Keywords: Silicates and yeast, saponins, growth, health status, shrimp *Litopenaeus vannamei*

ABSTRAK

Pada kajian ini, produk komersial yang berbasiskan kepada komponen alamiah silikat dan ragi (NSY) dan saponins yang berasal dari tumbuhan *Yucca schidigera* dan *Quillaja saponaria* (SBP) digunakan untuk mengevaluasi pengaruhu nutrisi yang dimiliki terhadap pertumbuhan dan status kesehatan dari udang putih *Litopenaeus vannamei*. Empat pakan yang bersifat iso-nitrogen dan iso-lipid diformulasikan dengan perlakuan 0%, 0.3% NSY, 0.2% SBP and 0.6% NSY. Setelah 60 hari, udang kemudian diambil sampelnya dan protokol standard untuk uji hemato-immunologi dilakukan. Laju pertumbuhan udang secara signifikan dipengaruhi oleh keberadaan NSY dan SBP, dimana inklusi NSY dan SBP memberikan pertumbuhan yang lebih baik dibandingkan kontrol. Selain itu, penggunaan NSY dan SBP secara signifikan meningkatkan jumlah total hemosit dan aktivitas lisozim pada udang perlakuan dibandingkan kontrol. Oleh karena itu, NSY dan SBP dapat digolongkan sebagai bahan baku fungsional pada pakan udang untuk meningkatkan pertumbuhan dan fungsi imun non-spesifik pada udang

Kata kunci: Silikat dan ragi, saponins, laju pertumbuhan, status kesehatan, udang *Litopenaeus vannamei*

INTRODUCTION

For years, most of the diet formulation for shrimp was designed to include approximately 20% fish meal (FM) due to its excellent sources of nutrients and mineral content (Sookying & Davis, 2011; Suárez *et al.,* 2009; Tacon & Akiyama, 1997). However, the combination of high prices and fluctuating supply of FM has resulted in higher feed costs; therefore it has grown the demand for the replacement of FM with plant-protein sources, which is relatively less expensive than FM (Novriadi & Davis, 2018; Hardy, 1996). Several alternative protein sources has been evaluated to replace FM and other animal proteins in shrimp feed diet formulation allowing increased levels of plant based proteins, including soybean meal and their advance products, cotton seed meal, and corn protein concentrate (Van Nguyen *et al.* 2018; Jatobá, *et al.* 2017; Richardson *et al* 2016; Bauer *et al.* 2012; Daiyong *et al.* 2009). Among alternative protein sources, processed soybean (*Glycine max*) possesses many qualities, including favorable amino acid profile, reasonable prices, widely available and highly digestible to be used as the most promising alternatives to replace FM in formulated diets (Lech & Reigh, 2012; Suárez *et al.,* 2009; Gatlin *et al.*, 2007, Amaya *et al.*, 2007; Davis & Arnold, 2000).

However, wider use of soy protein may be hindered with deficient levels of indispensable amino acids, anti-nutritional factors and poor palatability problem (Davis & Arnold, 2000). In addition, Liu *et al.* (2019) mentioned that due to the antigen protein, soy protein will likely induce a stress reaction in shrimp. Consequently, there is a need to properly adjust the diet composition or explore the use of functional dietary ingredients to enhance the efficacy of plant-based diets for shrimp. Functional ingredients could be defined as ingredients that provide healthy and economical benefits beyond the basic nutrition needs (Olmos *et al.,* 2011). A study from Nonwachai *et al.* (2010) showed that the use of heterotrophic algae as a source of essential fatty acids in feed designed with 40% soy protein significantly affected the immune response of shrimp and improved the resistance of shrimp against *Vibrio harveyi*. Furthermore, some functional ingredients, such as protein hydrolysates, prebiotics and herbal compounds, have been reported to possess healthy and nutraceutical properties and have been used as an additives to enhance the growth and positively modulate the immune system of shrimp (Servin *et al.,* 2021; Quinto *et al.,* 2018; Nguyen *et al.,* 2012). Under these circumstances, the use of functional ingredients could be considered as the most attractive innovation in aquafeed formulation to enhance the dietary nutrition of plant-based ingredients.

Other than formulated feed and ingredients used to produce the feed, healthy gut microbiota is essential to promote the use of plant-protein as the primary protein sources in aquafeed (Zhou *et al.,* 2018; Ringø *et al.,* 2016). The intestinal microbiota of shrimp may play a vital role in nutrient metabolism, pathogen resistance, supply vitamins and digestive enzymes when feeding with high carbohydrate diets (NRC, 2011). However, due to rapid passage rates, high load of plant-protein might prevent extensive microbial fermentation within the digestive system of shrimp (Shao *et al.,* 2019; NRC, 2011). The dietary effects on the use of fermented soybean meal, with lower anti-nutritional factors and better nutritional values than traditional soybean meal, on the intestinal microbiota has been examined by Shao *et al.* (2019) showing the ability of fermented soy to enhance the growth performance, but not significantly affect the intestinal microbiota composition of shrimp. Thus, the search on the functional ingredients that can improve the growth performance and contributing to a healthy intestinal microbiota condition as well as the haemato-immunological parameters in shrimp when using low FM and high inclusion levels of plant-protein sources are needed.

Recently, the growth and gut microbiota composition of shrimp fed with different inclusion levels of proprietary combination of all natural silicates and yeast components (NSY, PAQ-Gro™ Phibro, Teaneck, USA) has been evaluated (Servin *et al.,* 2021). The authors mentioned that the inclusion of 1% NSY into the diet formulated with 10% FM, 22% soybean meal (SBM), 18% poultry meal (PM) and 43% wheat flour (WF) as the primary ingredients were able to positively enhance the growth and modulating the gut microbiota of *L.vannamei*. Therefore, the purpose of this study is to focus on the functional effects of commercial NSY and SBP on the growth and haemato-immunological parameter of the Pacific white shrimp *L.vannamei*.

MATERIALS AND METHODS

Experimental diets

All diets were formulated to be similar in crude protein level (iso-nitrogenous) and crude lipid level (iso-lipidic) to contain 35% protein and 8% lipid as demonstrated in Table 1. In this growth trial, the control diet was designed with 10% fish meal (FM), 45% soybean meal (SBM), 17% wheat products (WP) and 8% corn protein concentrate (CPC) as the primary ingredients. Based on our internal assessment, diet 1 was produced by adding 0.3% of NSY, diet 2 was produced by incorporating 0.2% SBP, and diet 3 was produced by adding 0.6% of NSY into the control diet. All experimental diets were produced at the Main Center of Mariculture Development of Lampung using standard procedures for making shrimp feed and labeled as control diet, NSY 0.3; SBP 0.2; and NSY 0.6. Dry pellets were crumbled, packed in sealed bags, and stored in a freezer until use.

[1] High protein fish meal (Peru) supplied by Agri Permata Asia, Jakarta, Indonesia
[2] De-hulled solvent extract soybean meal, Bogor Ingredients, Indonesia
[3] Empyreal 75, Cargill, USA
[4] FKS Multi Agro, Jakarta, Indonesia
[5] PAQ-Gro™ Phibro, Teaneck, USA
[6] PAQ-Protex™ Phibro, Teaneck, USA
[7] Trace mineral premix (g/100 g premix): cobalt chloride, 0.004; cupric sulfate pentahydrate, 0.550; ferrous sulfate, 2.000; magnesium sulfate anhydrous, 13.862; manganese sulfate monohydrate, 0.650; potassium iodide, 0.067; sodium selenite, 0.010; zinc sulfate heptahydrate, 13.193; alpha-cellulose, 69.664.
[8] Vitamin premix (g/kg premix): thiamin·HCL, 4.95; riboflavin, 3.83; pyridoxine·HCL, 4.00; Ca-pantothenate, 10.00; nicotinic acid, 10.00; biotin, 0.50; folic acid, 4.00; cyanocobalamin, 0.05; inositol, 25.00; vitamin A acetate (500,000 IU/g), 0.32; vitamin D3 (1,000,000 IU/g), 80.00; menadione, 0.50; alpha-cellulose, 856.81

Table 1. Composition (% as is) of diets containing all-natural silicates and yeast components (NSY) and saponins obtained from *Yucca schidigera* and *Quillaja saponaria* (SBP) into the basal diet and fed to *L. vannamei* for 60 days

Ingredients (% *as is*)	Diet code			
	Control	NSY 0.3	SBP 0.2	NSY 0.6
Menhaden fishmeal[1]	10.00	10.00	10.00	10.00
Soybean meal[2]	45.00	45.00	45.00	45.00
Corn protein concentrate[3]	8.00	8.00	8.00	8.00
Menhaden fish oil[4]	5.64	5.64	5.64	5.64
Corn starch[4]	6.56	6.56	6.56	6.56
Yeast cell wall extract [5]	0.00	0.30	-	0.60
Natural feed additives[6]	-	-	0,20	-
Wheat products[4]	17.00	17.00	17.00	17.00
Mineral premix[7]	0.70	0.70	0.70	0.70
Vitamin premix[8]	1.90	1.90	1.90	1.90
CaP-dibasic[4]	2.50	2.50	2.50	2.50
Choline chloride[4]	0.20	0.20	0.20	0.20
KP dibasic[4]	1.50	1.50	1.50	1.50
Proximate analysis (% as is)[8]				
Crude protein	35.58	35.88	35.14	36.11
Lysine	1.98	1.97	2.02	2.04
Methionine	0.80	0.82	0.78	0.84
Moisture	7.68	7.82	7.51	7.59
Crude fat	8.13	8.38	8.72	8.95
Crude fiber	3.56	3.49	3.63	3.76
Ash	6.15	6.90	5.64	6.30

[9] Analysis conducted by the SUA Integrated Fish Farm, Bogor Agricultural University, West Java, Indonesia

Growth trial and feeding management

The growth trials were conducted at the PT. Batam Dae Hae Seng research station (Batam, Indonesia). Pacific white shrimp post larvae (PL) were obtained from PT. Prima Akuakultur Lestari (Kalianda, Lampung, Indonesia) and were acclimatized to the culture system. Post-larvae were fed with a commercial feed (Evergreen Feed, Lampung, Indonesia) for three weeks until they reached the suitable size. Shrimp (4.24 ± 0.03 g initial mean weight) were randomly distributed into 24 tanks with size of 70 × 35 × 40 cm (98 L) per aquaria tank. Six replicate groups of shrimp were offered experimental diets using nutrition research standard protocol for 70 days and fed by hand four times daily at 07:00, 11:00, 15:00 and 20:00. Feed inputs were pre-programmed assuming the normal growth of shrimp and feed conversion ratio of 1.5. Daily allowances of feed were adjusted based on observed feed consumption, weekly counts of the shrimp and mortality. Uneaten feed, feces, and molts were removed by siphoning the aquaria tank prior to the first feeding.

Water quality and growth sampling

Dissolved oxygen (DO), pH, water temperature and salinity were measured four times daily using Aqua TROLL 500 Multiparameter Sonde instrument and connected to AquaEasy apps (Bosch, Singapore). Total ammonianitrogen (TAN), nitrate and nitrite were measured once a week by using absorption spectrophotometry (DR890, HACH, USA). At the end of feeding period, all shrimp were grouped and individually weighed to calculate the final biomass, final weight, percentage weight gain (PWG), feed conversion ratio (FCR), percentage survival (SR) and thermal unit growth coefficient (TGC) as follows:

$$PWG = \frac{\text{(average individual final weight – average individual initial weight)}}{\text{(average individual initial weight)}} \times 100$$

$$FCR = \frac{\text{Feed consumption (g)}}{\text{Weight gain (g)}}$$

$$SR = \frac{\text{Final number of fish}}{\text{Initial number of fish}} \times 100$$

$$TGC = \frac{PBW^{1/3} - IBW^{1/3}}{\sum TD} \times 100$$

Where FBW is final body weight, IBW is initial body weight, T is water temperature (^{0}C) and D is number of trial days

Total haemocytes count

At the end of growth trial, hemolymph was sampled from two intermolt shrimp per tank or ten shrimp per treatment and total hemocytes count was determined. Hemolymph (100 µL) of individual shrimp was withdrawn from the pleopod base of the second abdominal segment with a sterile 1-mL syringe (25 G × 13 mm needle). Before hemolymph extraction, the syringe was loaded with a precooled (4°C) solution (10%-EDTA, Na2) used as an anticoagulant. The haemolymph with anti-coagulant solution was diluted in 150 µL of formaldehyde (4%) and then 20 µL was placed on a hemocytometer (Neubauer) to determine the total haemocytes count (THC) using an optical microscope (Olympus, DP72).

Lysozyme activity analysis

Lysozyme activity was measured using a lysozyme detection kit (Sigma-Aldrich, Cat. no. LY0100) according to the manufacturer's instruction. The results of lysozyme activity were defined by the lysis of the *Micrococcus lysodeikticus* cells. The reactions were conducted at 25 °C and absorbance at 450 nm was measured on the ultraviolet/visible spectrophotometer (Perkin Elmer, Lambda XLS, USA)

$$\text{Lysozyme activity } \left(\frac{\text{Units}}{\text{mL}}\right) = \frac{(\Delta A450 / \text{minTest} / \Delta A450 \text{ minBlank})(df)}{(0.001)\,(0.03)}$$

df = dilution factor
0.001 = ΔA_{450} as per the unit definition
0.03 = Volume (in milliliters) of enzyme solution

Whole-body protein level and protein retention rate

Upon termination of the trial, four shrimp from each tank or twenty-four shrimp per treatment were randomly sampled and stored at -60 ^{0}C for body composition analysis. Prior to the protein analysis, dried whole shrimp were rigorously blended and chopped in a mixer according to the standard methods established by Association of Official Analytical Chemists (AOAC, 1990). Protein contents of whole shrimp body were analyzed by using Kjeldahl method and conducted at PT. Angler BioChem Lab (Surabaya, East Java, Indonesia). The protein retention rate (%) were calculated using formula as follows:

$$\text{Protein retention rate (\%)} = \frac{\text{(final weight*final protein)-(initial wiight*ini.protein)}}{\text{Intotal protein intake (dry matter)}} \times 100$$

Statistical analysis

All data were analyzed using one-way analysis of variance to determine the significant difference (P<0.05) among the treatment means followed by the Tukey's multiple comparison test to determine difference between treatments means in each trial. The pooled standard errors were used across all the growth parameters as the variance of each treatment is the same. Statistical analyses were conducted using SAS system (V9.4, SAS Institute, Cary, NC, USA).

RESULTS AND DISCUSSION

Results

Water quality

As shown in Table 2, the overall mean and standard deviation of morning and afternoon pH (7.68 ± 0.12 and 7.81 ± 0.29), salinity (25.24 ± 2.02–25.93 ± 2.62‰), water temperature (27.92 ± 0.44–29.34 ± 0.65 ^{0}C) and dissolved oxygen (5.71 ± 0.35–5.82 ± 0.62 mg/L) together with ammonia (0.09 ± 0.06 mg TAN/L) and nitrate (28.77 ± 5.62 mg NO_2-N/L) are still within the optimal range for *L. vannamei.*

Growth performance

Based on the growth performance and survival of Pacific white shrimp L. *vannamei* fed with the experimental diet summarized in Table 3, the use of NSY and SBP were able to significantly enhance the growth of *L. vannamei* compared to the control group. With respect to individual growth, both treatments displayed significantly higher final mean weight with better FCR compared to the control group. Despite lack of statistical significance, numerically, the survival of shrimp treated with NSY and SBP increased by 5.06–6.33% compared to the control group. The thermal growth coefficient (TGC) and weight gain (WG) of shrimp treated both with NSY and SBP were significantly higher than control group (P<0.05). Protein level and protein retention (%) of pacific white shrimp, *Litopenaeus vannamei* fed diets containing different levels of NSY and SBP are displayed in Figure 1. Supplementation of NSY and SBP did not affect the protein level of whole body of the shrimp. However, biologically, diet supplemented with 0.2% of SBP showed a higher protein level and protein retention rate (%) compared to other treatments which is also correlates with the growth of the shrimp.

Table 2. Water quality data during the grow-out phase of the experiment. Data were presented as mean± standard deviation (range).

Time	Parameter					
	Temperature (˚C)	D.O (mg/L)	pH	Salinity (‰)	Ammonia (mg/L)	Nitrate (mg/L)
AM	27.92 ± 0.44	5.71 ± 0.35	7.68 ± 0.12	25.24 ± 2.02	0.09 ± 0.06	28.77 ± 5.62
PM	29.34 ± 0.65	5.82 ± 0.62	7.81 ± 0.29	25.93 ± 2.62		

Table 3. Growth performance of Pacific white shrimp *Litopenaeus vannamei* (Mean initial weight 4.24 ± 0.03 g) fed experimental diets for 60 d. Values represent the mean of six replicates. Results in the same columns with different superscript letter are significantly different (P<0.05) based on analysis of variance followed by Tukey's multiple comparison test.

Diet code	Final biomass (g)	Final mean weight (g)	Survival (%)	WG (%)	FCR[2]	TGC[3]
Control diet	190.24^b	14.47^b	87.78^a	241.02^b	2.17^a	0.0475^b
NSY 0.3	215.55^{ab}	15.58^a	92.22^a	268.20^a	2.00^b	0.0511^a
SBP 0.2	221.64^a	15.83^a	93.33^a	273.71^a	1.97^b	0.0519^a
NSY 0.6	219.86^{ab}	15.75^a	93.33^a	271.86^a	1.98^b	0.0516^a

Hemato-immunological response

The effect of adding NSY and SBP on the haemato-immunological parameters of shrimp are displayed in Figure 2 and Figure 3. The group of shrimp treated with 0.2% SBP and 0.6% NSY had the highest total haemocytes count (THC) compared to the control group. This also correlates with the lysozyme activity, whereas the highest activity was found in shrimp treated with 0.2% SBP followed with the supplementation of 0.3 and 0.6% NSY.

Discussion

In intensive culture system, more attention is being given to functional ingredients, additives and supplements to provide nutritional support for defense mechanisms enhancement, response to the degradation of water quality, maintain the energy requirement and optimize the metabolism function of shrimp (Delgado *et al.,* 2021; Servin *et al.,* 2021; Niu *et al.,* 2019; Zhu *et al.,* 2019; Cruz-Suárez *et al.,* 2009; Li *et al.,* 2007; Ochoa-Solano *et al.,* 2006). At the same time, the use of

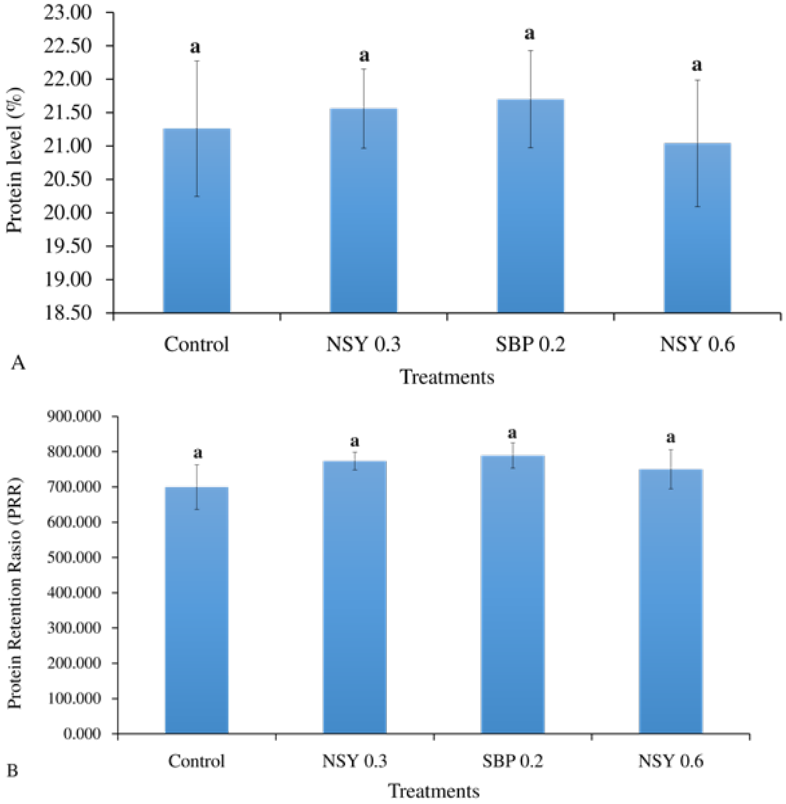

Figure 1. Protein level (A) and protein retention rate (%) (B) of whole body of Pacific white shrimp *Litopenaeus vannamei* fed experimental diets for 60 d. Values represent the mean of six replicates.

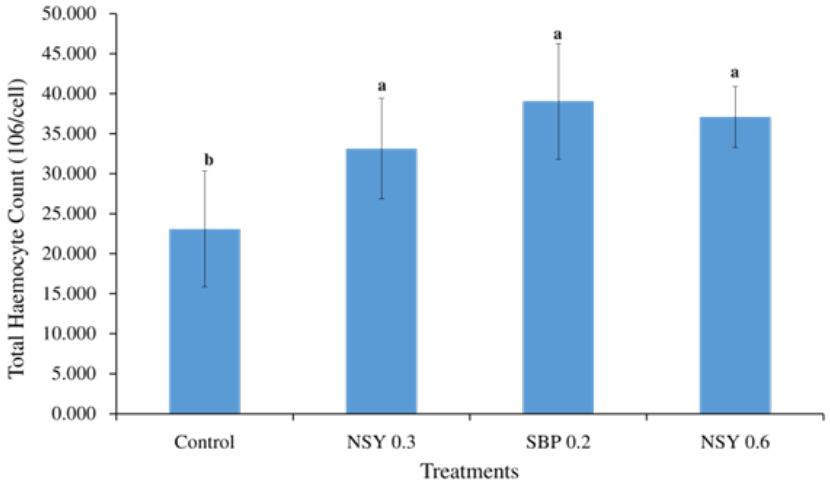

Figure 2. Total hemocyte count of Pacific white shrimp *Litopenaeus vannamei* (10^6 cell/mL) at the end of growth trial. Values represent the mean of six replicates.

functional ingredients to partially or completely replace the use of fish meal (FM) could provide economic benefits with well-balanced and less expensive diets, as well as increasing the productivity of shrimp farms (Olmos *et al.,* 2011; Bautista-Teruel *et al.,* 2003). The present study evaluated the effect of commercial natural silicates and yeast (NSY) and saponins from *Yucca schidigera* and *Quillaja saponaria* plants (SBP) as feed additives on the growth performance and health condition of shrimp.

Based on the growth performance and survival of Pacific white shrimp *L. vannamei* fed with the experimental diet summarized in Table 3, the use of a combination of all-natural silicates and yeast components (NSY) and natural feed additive containing saponin blend (SBP) significantly increase final biomass (FB), average individual weight (AIW), thermal growth coefficient (TGC) and weight gain (WG). With reference to the use of NSY, the growth results are in line with the recent study from Servin *et al.* (2021) in which shrimp displayed significantly better AIW, WG and FB with the administration of 1.0 % NSY into the diet (10% FM, 22% SBM and 43% WF) in comparison to the group of shrimp that were not receiving NSY into their diet. The same author observes that the direct stimulation of the immune system by the polysaccharides-rich feed contained in 1.0 % NSY diet appears to be directly related to the superior growth of shrimp in this group compared with lower dose of NSY (0.5 %) and control group. Interestingly, in this study, the inclusion level of NSY also played a significant role in enhancing the growth of shrimp. Despite no significant statistical differences, numerically, it was observed that FB, AIW, TGC, and WG improved with the use of 0.6% NSY compared to 0.3% NSY. The synergistic effect between various prebiotics and immunostimulant contain within the product could influence the physiological function of shrimp and eventually able to support the optimum growth of shrimp.

The addition of 0.2% SBP had the most influence on the growth of the shrimp. Studies on the use of natural compounds extracted from *Yucca schidigera* and *Quillaja saponaria* has been evaluated resulting in better growth performance of striped catfish, *Pangasianodon hypophthalmus* (Güroy *et al.,* 2016), nile tilapia *Oreochromis niloticus* (Angeles *et al.,* 2017) and shrimp, *Litopenaeus vannamei* (Hernández-Acosta *et al.,* 2016). According to Acosta *et al.* (2019), saponins extracted from *Yucca schidigera* and *Quillaja saponaria* have proven to be very promising ingredients for aquafeed as natural growth promoters. Furthermore, studies using high levels of steroidal compounds from branches and leaves of *Yucca schidigera* have shown promising activity in the adsorption of harmful compounds such as ammonia (Fayed *et al.,* 2019). Thus, the use of SBP as a feed additive during shrimp production could provide economic benefits in terms of productivity and efficiency.

Other than growth performance, this study evaluated the protein retention rate and protein level in the whole body of the shrimp as the effect of NSY and SBP addition into the diet. Despite

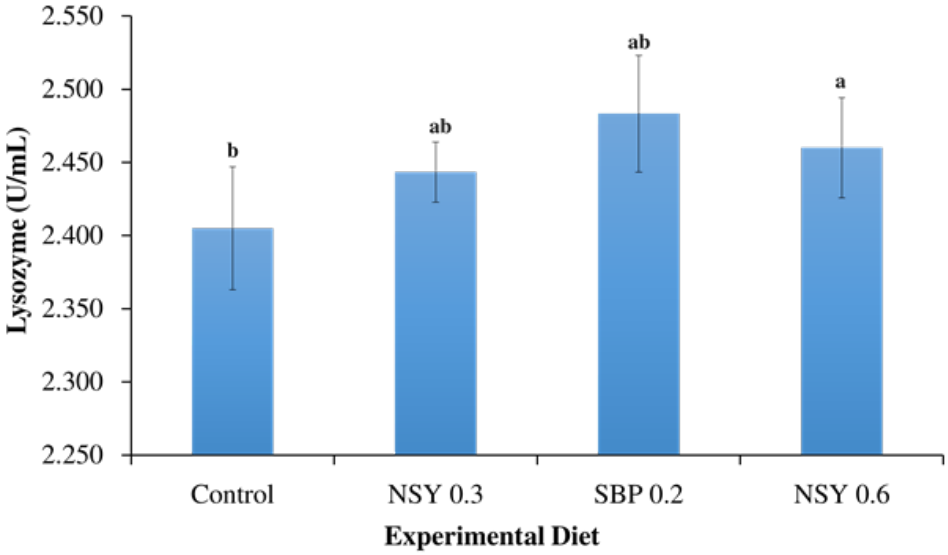

Figure 3. Lysozyme activity of Pacific white shrimp *Litopenaeus vannamei* (U/mL) at the end of growth trial. Values represent the mean of six replicates.

no significant differences in terms of statistical analysis, the findings showed higher numerical value of protein level and retention rate (%) in shrimp treated with NSY and SBP compared to the control treatment. A similar effect was also found in a study with striped catfish *Pangasianodon hypophthalmus*, where the dietary inclusion of *Yucca schidigera* and *Quillaja saponaria* at all levels investigated did not affect whole body proximate composition of the fish (Güroy *et al*., 2016). Rates and efficiencies in nutrient deposition are governed by dietary factors, including nutrient balance and nutrient utilization, as well as biological factors such as genetics and the physiological state of the animal (NRC, 2011). Since all shrimps were maintained in similar culture environments and the diets were designed to be iso-nitrogenous in this study, this could explain the comparable protein levels in the whole body composition of the shrimp among the dietary treatments.

The potential application of saponins from *Yucca schidigera* and *Quillaja saponaria* to enhance the passive immunization response has been well described in Cheeke (2000). The strong immunoadjuvant activity of *Q. saponaria* and its derivatives makes them ideal substances for the development of vaccines (Rajput *et al*., 2007; Sjölander *et al*., 2001; Cheeke, 2000) In aquaculture, the presence of yucca in the diet positively affects the hematological and immunological response of European seabass, *Dicentrarchus labrax* (Fayed *et al*., 2019). Furthermore, the lysozyme activity of juvenile nile tilapia *Oreochromis niloticus* increases as the inclusion of dietary yucca increases up to 0.1 % in the diet formulation (Njagi *et al*., 2017). However, the same authors also reported that the use of dietary yucca higher than 0.1% did not show any further increase in lysozyme activity.

In this study, the haemocytes counts and lysozyme activity were higher in the group of shrimp that fed with commercial NSY and SBP. The elevation of total haemocytes counts and lysozyme activity in shrimp might be due to the immunomodulatory function of commercial NSY and SBP as well as the presence of saponins that are able to enhance the non-specific immune system of shrimp. Increased percentage the use of 0.2% SBP provided better results for lysozyme activity compared to the control group. Invertebrates lack the complexity of the adaptive immune system compared to vertebrates. This means that invertebrates, due to the absence of "true" lymphocytes and functional antibodies rely solely on the innate immune system that are largely based on haemocytes (Smith, 1991). There are three types of haemocytes in shrimp, namely: hyaline cells, semi-granular cells, and granular cells, mainly responsible for phagocytic activity and synthesis of melanin which is a powerful antimicrobial component for shrimp (Söderhäll & Cerenius, 1998; 1992; Söderhäll & Smith, 1983).

CONCLUSION

Results in this study confirm the beneficial effects of all-natural silicates and yeast components and saponins obtained from *Yucca schidigera* and *Quillaja saponaria* as a functional property in shrimp feed formulation. Under the conditions of the present study, the inclusion of 0.3 and 0.6% commercial complex of all-natural silicates and yeast components and 0.2 % saponin blend extracted from *Yucca schidigera* and *Quillaja saponaria* could be used to enhance the growth and health status of Pacific white shrimp *L. vannamei*.

ACKNOWLEDGEMENTS

Phibro Animal Health Corporation provided the research funding and donated the commercial product analyzed in this study. Special thanks to Raja Ali Haji Maritime University students for their help in sampling program. The authors would also like to extend the gratitude to those who have taken the time to critically review this manuscript as well as those who helped in supporting this research. Mention of trademark or proprietary product does not constitute an endorsement of the product by the Jakarta Technical University of Fisheries and does not imply its approval to the exclusion of other products that may also be suitable.

REFERENCES

Acosta R, Rosen Y, Ariav R. 2019. The use of saponins in aquaculture. International Aquafeed. August 2019. Perendale Publishers, Ltd., pp. 32–37.

Amaya E, Davis DA, Rouse DB. 2007. Alternative diets for the Pacific white shrimp *Litopenaeus vannamei*. Aquaculture 262: 419–425.

Angeles Jr IP, Gallego LM, Navarro MAM, Chien YH. 2017. Dietary effects of *Quillaja saponaria* and *Yucca schidigera* extract on rearing performance of Nile tilapia *Oreochromis niloticus* L. and its antioxidant capacity and metabolic response following hypoxic stress. International Journal of Agricultural Technology 13: 2249–2266.

Bauer W, Prentice-Hernandez C, Tesser, MB, Wasielesky Jr W, Poersch LH. 2012. Substitution of fishmeal with microbial floc meal and soy protein concentrate in diets for the pacific white shrimp *Litopenaeus vannamei*. Aquaculture 342: 112–116.

Bautista-Teruel MN, Eusebio PS, Welsh TP. 2003. Utilization of feed pea, *Pisum sativum,* meal as a protein source in practical diets for juvenile tiger shrimp, *Penaeus monodon*. Aquaculture 225: 121–131.

Cheeke PR. 2000. Actual and potential applications of *Yucca schidigera* and *Quillaja saponaria* saponins in human and animal nutrition. In Saponins in food, feedstuffs and medicinal plants (pp. 241–254). Springer, Dordrecht.

Cruz-Suárez LE, Tapia-Salazar M, Nieto-López MG, Guajardo-Barbosa C, Ricque-Marie D. 2009. Comparison of *Ulva clathrata* and the kelps *Macrocystis pyrifera* and *Ascophyllum nodosum* as ingredients in shrimp feeds. Aquaculture Nutrition 15: 421–430.

Delgado E, Valles-Rosales DJ, Flores NC, Reyes-Jáquez D. 2021. Evaluation of fish oil content and cottonseed meal with ultralow gossypol content on the functional properties of an extruded shrimp feed. Aquaculture Reports 19: 100588.

Daiyong W, Yuantu Y, Baotong Z. 2009. Effects of cotton seed meal and rapeseed meal on growth performance, non-specific immune indexes and body compositions of *Litopenaeus vannamei* [J]. China Feed 23: 12.

Davis DA, Arnold C. 2000. Replacement of fish meal in practical diets for the Pacific white shrimp, *Litopenaeus vannamei*. Aquaculture 185: 291–298

Fayed WM, Khalil RH, Sallam GR, Mansour AT, Elkhayat BK, Omar EA. 2019. Estimating the effective level of *Yucca schidigera* extract for improvement of the survival, haematological parameters, immunological responses and water quality of European seabass juveniles *Dicentrarchus labrax*. Aquaculture Reports 15: 100208.

Gatlin DM, Barrows FT, Brown P, Dabrowski K, Gaylord TG, Hardy RW, Herman E, Hu G, Krogdahl Å, Nelson R. 2007. Expanding the utilization of sustainable plant products in aquafeeds: a review. Aquaculture research 38: 551–579

Güroy B, Mantoğlu S, Merrifield DL, Guroy D. 2016. Effects of dietary Nutrafito Plus on growth, haemotological parameters and total ammonia-nitrogen excretion of juvenile striped catfish *Pangasianodon hypophthalmus*. Aquaculture Research 47: 1770–1777.

Hardy RW. 1996. Alternate protein sources for salmon and trout diets. Animal Feed Science and Technology 59: 71–80.

Hernández-Acosta M, Gutiérrez-Salazar GJ, Guzmán-Sáenz FM, Aguirre-Guzmán G, Alvarez-González CA, López-Acevedo EA, Fitzsimmons K. 2016. Los efectos de *Yucca schidigera* y *Quillaja saponaria* sobre el crecimiento y actividad enzimática de camarones juveniles de *Litopenaeus vannamei cultivados* a baja salinidad. Latin American Journal of Aquatic Research 44: 121–128.

Jatobá A, Vieira FD N, Silva BCD, Soares M, Mouriño J L P, Seiffert W Q. 2017. Replacement of fishmeal for soy protein concentrate in diets for juvenile *Litopenaeus vannamei* in biofloc-based rearing system. Revista Brasileira de Zootecnia 46: 705–713.

Lech GP, Reigh RC. 2012. Plant products affect growth and digestive efficiency of cultured Florida pompano *Trachinotus carolinus* fed compounded diets. PLoS One, 7: e34981.

Li P, Burr G S, Gatlin III D M, Hume M E, Patnaik S, Castille F L, Lawrence A L. 2007. Dietary supplementation of short-chain fructooligosaccharides influences gastrointestinal microbiota composition and immunity characteristics of Pacific white shrimp, *Litopenaeus vannamei*, cultured in a recirculating system. The Journal of Nutrition 137: 2763–2768.

Nonwachai T, Purivirojkul W, Limsuwan C, Chuchird N, Velasco M, Dhar A K. 2010. Growth, nonspecific immune characteristics, and survival upon challenge with *Vibrio harveyi* in Pacific white shrimp *Litopenaeus vannamei* raised on diets containing algal meal. Fish & Shellfish Immunology 29: 298–304.

Nguyen HTM, Pérez-Gálvez R, Bergé JP. 2012. Effect of diets containing tuna head

hydrolysates on the survival and growth of shrimp *Penaeus vannamei*. Aquaculture 324: 127–134.

Niu J, Xie JJ, Guo TY. Fang HH, Zhang YM, Liao SY, Xie SW, Liu YJ, Tian LX. 2019. Comparison and evaluation of four species of macro-algaes as dietary ingredients in *Litopenaeus vannamei* under normal rearing and WSSV challenge conditions: effect on growth, immune response, and intestinal microbiota. Frontiers in Physiology 9: 1880.

Njagi GW, Lee S, Won S, Hong J, Hamidoghli A, Bai SC. 2017. Effects of dietary Yucca meal on growth, haematology, non-specific immune responses and disease resistance of juvenile Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758). Aquaculture Research 48: 4399–4408.

Ochoa-Solano JL, Olmos-Soto J. 2006. The functional property of *Bacillus* for shrimp feeds. Food Microbiology 23: 519–525.

Olmos J, Ochoa L, Paniagua-Michel J, Contreras R. 2011. Functional feed assessment on *Litopenaeus vannamei* using 100% fish meal replacement by soybean meal, high levels of complex carbohydrates and *Bacillus* probiotic strains. Marine Drugs 9: 1119–1132.

Novriadi R, Davis DA. 2018. Research update: Development of plant-based diets for Florida pompano *Trachinotus carolinus*. Aquacultura Indonesiana 19: 47–56.

NRC (National Research Council). 2011. Nutrient requirements of fish and shrimp. National Academy Press. Washington, D.C., USA.

Quinto BPT, Albuquerque J V, Bezerra RS, Peixoto S, Soares R. 2018. Replacement of fishmeal by two types of fish protein hydrolysate in feed for postlarval shrimp *Litopenaeus vannamei*. Aquaculture Nutrition 24: 768–776.

Rajput ZI, Hu SH, Xiao CW, Arijo AG. 2007. Adjuvant effects of saponins on animal immune responses. Journal of Zhejiang University Science 8: 153–161.

Richardson CM, Siccardi AJ, Palle SR, Campbell LM, Puckhaber L, Stipanovic RD, Wedegaertner TC, Rathore KS, Samocha TM. 2016. Evaluation of ultra-low gossypol cottonseed and regular glandless cottonseed meals as dietary protein and lipid sources for *Litopenaeus vannamei* reared under zero-exchange conditions. Aquaculture Nutrition 22: 427–434.

Ringø E, Zhou Z, Vecino JG, Wadsworth S, Romero J, Krogdahl Å, Olsen RE, Dimitroglou A, Foey A, Davies S, Owen M, Lauzon HL, Martinsen LL, De Schryver P, Bossier P, Sperstad S, Merrifield DL. 2016. Effect of dietary components on the gut microbiota of aquatic animals. A never-ending story?. Aquaculture Nutrition 22: 219–282.

Servin Arce K, de Souza Valente C, do Vale Pereira G, Shapira B, Davies S J. 2021. Modulation of the gut microbiota of Pacific white shrimp (*Penaeus vannamei* Boone, 1931) by dietary inclusion of a functional yeast cell wall-based additive. Aquaculture Nutrition 27: 1114–1127.

Shao J, Wang B, Liu M, Jiang K, Wang L, Wang M. 2019. Replacement of fishmeal by fermented soybean meal could enhance the growth performance but not significantly influence the intestinal microbiota of white shrimp *Litopenaeus vannamei*. Aquaculture 504: 354–360.

Smith VJ. 1991. Invertebrate immunology: phylogenetic, ecotoxicological and biomedical implications. Comparative Haematology International 1: 61–76.

Söderhäll K, Cerenius L. 1992. Crustacean immunity. Annual Review of Fish Disease 2: 3–23.

Söderhäll K, Cerenius L. 1998. Role of the prophenoloxidase-activating system in invertebrate immunity. Current Opinion in Immunology 10: 23–28.

Söderhäll K, Häll L. 1984. Lipopolysaccharide-induced activation of prophenoloxidase activating system in crayfish haemocyte lysate. Biochimica et Biophysica Acta 797: 99–104.

Söderhäll K, Smith VJ. 1983. Separation of the haemocyte populations of *Carcinus maenas* and other marine decapods, and prophenoloxidase distribution. Developmental dan Comparative Immunology 7: 229–239.

Sjölander A, Drane D, Maraskovsky E, Scheerlinck J P, Suhrbier A, Tennent J, Pearse M. 2001. Immune responses to ISCOM® formulations in animal and primate models. Vaccine 19: 2661–2665.

Sookying D, Davis DA. 2011. Pond production of Pacific white shrimp *Litopenaeus vannamei* fed high levels of soybean meal in various combinations. Aquaculture 319: 141–149.

Suárez JA, Gaxiola G, Mendoza R, Cadavid S,

Garcia G, Alanis G, Suáreza A, Faillace J, Cuzon G. 2009. Substitution of fish meal with plant protein sources and energy budget for white shrimp *Litopenaeus vannamei* (Boone, 1931). Aquaculture 289: 118–123.

Tacon AGJ, Akiyama DM. 1997. Feed ingredients for crustaceans. In: D'Abramo, L.R., Conklin, D.E., Akiyama, D.M. (Eds.), Crustacean Nutrition. The World Aquaculture Society, Baton Rouge, LA, USA, pp. 411–472.

Van Nguyen N, Hoang L, Van Khanh T, Duy Hai P, Hung L T. 2018. Utilization of fermented soybean meal for fishmeal substitution in diets of Pacific white shrimp (*Litopenaeus vannamei*). Aquaculture Nutrition 24: 1092–1100.

Zhang M, Sun Y, Chen K, Yu N, Zhou Z, Chen L, Zhenyu D, Li E. 2014. Characterization of the intestinal microbiota in Pacific white shrimp, *Litopenaeus vannamei*, fed diets with different lipid sources. Aquaculture 434: 449–455.

Zhou Z, Ringø E, Olsen RE, Song SK. 2018. Dietary effects of soybean products on gut microbiota and immunity of aquatic animals: a review. Aquaculture Nutrition 24: 644–665.

Zhu T, Morais S, Luo J, Jin M, Lu Y, Le Y, Zhou Q. 2019. Functional palatability enhancer improved growth, intestinal morphology, and hepatopancreas protease activity, replacing squid paste in white shrimp, *Litopenaeus vannamei*, diets. Journal of the World Aquaculture Society 50: 1064–1077.