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Effects of nano-scale nutrients supplement on natural productivity of *Thalassiosira* sp. and growth performance of Pacific white shrimp, *Litopenaeus vannamei*, reared under intensive conditions using concrete tank culture system

Efek suplementasi nano-nutrien terhadap produktivitas alamiah *Thalassiosira* sp. dan pertumbuhan udang *Litopenaeus vannamei* yang dipelihara dalam kondisi intensif menggunakan sistem pemeliharaan bak beton

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ABSTRACT

The aim of this study was to evaluate the use of unique mixture of nano-nutrient to extent the growth of diatom *Thalassiosira* sp. and the effect to the water quality, growth performance, and protein composition on the whole body of the Pacific white shrimp *Litopenaeus vannamei*. There are four treatments with four replicates per treatment with the use of commercial nano-nutrients (Aquaritin Aquaculture or AA) namely: (1) 0.70 mg/L; (2) 0.525 mg/L, (3) 0.35 mg/L, and (4) without any AA application, but included standard application of using urea and NPK fertilizers to enhance the growth of diatom. The use of AA was successful to trigger the growth of *Thalassiosira* sp. Group of shrimp treated with 0.70 mg/L had better growth rate. Results of feeding trial indicated that adding AA could also improve the biomass, final mean weight, survival, percentage weight gain, and better feed utilization in terms of FCR. The addition of AA to enhance the growth of *Thalassiosira* sp. also provides a beneficial impact to the protein composition in whole body of shrimp. Biologically, the protein composition in the whole body of shrimp treated with 0.7 mg/L was higher. The findings from this study showed that the addition of commercial nano-nutrient could enhance the growth of *Thalassiosira* sp. and led to better growth of shrimp cultured in concrete tank

Key words: Growth, *Litopenaeus vannamei*, nano-nutrients, protein composition, *Thalassiosira* sp**.**

ABSTRAK

Tujuan penelitian yaitu mengevalusi penggunaan campuran unik nano-nutrien untuk meningkatkan pertumbuhan diatom *Thalassiosira* sp. dan pengaruhnya terhadap kualitas air, laju pertumbuhan, dan komposisi protein pada tubuh udang *L. vannamei*. Terdapat empat perlakuan dan empat ulangan per perlakuan dengan penggunaan nano-nutrisi komersial (Aquaritin Aquaculture atau AA) yaitu: (1) 0,70 mg/L; (2) 0,525 mg/L; (3) 0,35 mg/L, dan (4) tanpa pemberian AA, tetapi menggunakan penerapan standar pupuk urea dan NPK untuk pertumbuhan diatom. Penggunaan AA berhasil memicu pertumbuhan *Thalassiosira* sp. Kelompok udang yang diberi perlakuan 0,70 mg/L memiliki laju pertumbuhan yang lebih baik. Hasil uji coba pemberian pakan menunjukkan bahwa penambahan AA juga dapat meningkatkan biomassa, bobot rata-rata akhir, kelangsungan hidup, persentase pertambahan bobot dan pemanfaatan pakan yang lebih baik dalam hal FCR. Penambahan AA untuk meningkatkan pertumbuhan *Thalassiosira* sp. juga memberikan dampak yang menguntungkan bagi komposisi protein di seluruh tubuh udang. Secara biologis komposisi protein di tubuh udang dengan perlakuan 0,70 mg/L lebih tinggi. Hasil dari kajian ini menunjukkan bahwa penambahan nano-nutrien komersial dapat meningkatkan pertumbuhan *Thalassiosira* sp. dan mendorong pertumbuhan udang bada sistem budidaya di bak beton.

Kata kunci: Komposisi protein, *Litopenaeus vannamei*, nano-nutrien, pertumbuhan, *Thalassiosira* sp.

INTRODUCTION

Shrimp production system could be considered as the most promising food production sector providing protein-rich supplements for human consumption and constitutes as the valuable internationally traded aquaculture commodity worldwide (Kumar & Engle, 2016; Samerwong *et al.,* 2018; Lee *et al*., 2019). Recently, shrimp culture system has changed from extensive, traditional, and small-scale productions to an intensive system that fully support with technology, and large-scale production system to fulfill the market demand (Reis *et al.,* 2020; Bardera *et al.,* 2020; Zulkarnain *et al.,* 2020; Soares *et al.,* 2021). This changed followed by the use of high stocking density ranging from 110–500 shrimp/m^2 for intensive system and >500 shrimp/m^2 for supra-intensive farming system (Haslun *et al*., 2012; Zulkarnain *et al*., 2020). There are advantages and disadvantages of using (supra) intensive technology. According to Samocha (2019), high stocking density of shrimp in intensive system will lead to greater yields and more efficient in the use of culture environment. However, high inputs of nutrients and limitation on water exchange will create water quality problems that do not always arise in traditional or semi-intensive farming system (Anh *et al*., 2010; Suantika *et al.*, 2015; Jescovitch *et al*., 2018; Samocha, 2019). Furthermore, with regards to high stocking density, one of the strategies that need to be considered in order to increase the production efficiency is the use of microalgae including diatoms and green algae, as a complemented live feed in shrimp culture system (Ju *et al.,* 2009; Samocha *et al.,* 2015; Niu *et al.,* 2018).

The presence of microalga community, especially diatoms, are essential and play an important role to enhance the quality of feed due to their high nutritional value and can contribute with essential amino acids and highly unsaturated fatty acids (HUFA) (Ju *et al*., 2009; Godoy *et al.,* 2012; Jamali *et al*., 2015; Martins *et al.,* 2016; de Abreu *et al*., 2019). In terms of productivity, diatoms are thought to contribute as much as 45% of the total oceanic primary production (Mann, 1999) and the diatom-dominated floc culture has been considered as a good source of nutrition that could enhance the growth of the shrimp (da Silva *et al.,* 2013). Moreover, biomass of microalga *Thalassiosira pseudonana* has been considered as an essential feed for white shrimp seeding productions due to the their fatty acid, protein, carbohydrates content and large variety of minerals that can fulfill the specific nutrient requirement of *Litopenaeus vannamei* at the early culture stage (Van Nguyen, 2018). These studies suggest the potential benefit of providing diatoms to increase the production efficiency. Unfortunately, the growth of diatoms *Thalassiosira* sp. also affected by salinity and other environmental factors within the culture environment (García *et al*., 2012). Therefore, it is important to develop better strategy to enhance the growth of *Thalassiosira* sp.

Aquaritin Aqua, a commercial nano nutrients, is a unique mixture of nutrient inputs designed at nano scale targeted for sustained growth of diatoms, esp. *Thalasissoria* sp.. The inter nutrient ratios and sizes in Aquaritin Aqua are designed to obviate any possibility of nutrient toxicity. Sustained enhancement in diatom population provides live feed to the microscopic shrimp larvae, which develops their raptorial behavior and inherent autolysis system. The growth of diatoms deliver many benefits, including enhances the survival rate (SR), reduces feed conversion ratio (FCR) and causes significant reduction in blue green algae. Prolific photosynthesis by sustained population of diatoms also enhances the dissolved oxygen levels across the water-body that helps in cutting down aerator running hours. There is also limited information about the effect of diatoms *Thalassiosira* sp. to enhance the growth of Pacific white shrimp *Litopenaeus vannamei.* Therefore, the aim of the present study was to evaluate the extent growth of diatoms *Thalassiosira* sp. triggered by Aquaritin Aqua nano scale nutrients and their effect to the clarity and quality of water, growth performance of Pacific white shrimp *Litopenaeus vannamei*, and protein composition on the whole body of the shrimp.

MATERIALS AND METHODS

Algae culture

The pure culture of *Thalassiosira* sp. were obtained from Batam Dae Seng Indonesia (Batam, Riau Island province, Indonesia). Prior to stocking, diatom were held for two weeks in a 5,000 m^2 acclimation trough in seawater, filled with natural seawater (29–32 g/L). This water fertilized once with NO_3, PO_4, Fe, and other trace minerals. At start of the trial, the algae were harvested, cropped, and restocked at pre-determined treatment densities.

Growth trial

This study was performed at the Batam Dae Seng Indonesia (Batam, Riau Island province, Indonesia). Post larvae of Pacific white shrimp *Litopenaeus vannamei* (PL8 weighing 0.03–0.05 g) were obtained from PT Maju Tambak Sumur hatchery in Kalianda, Lampung, Indonesia. At the start of the production trial, the culture ponds were prepared with the addition of *Thalassiosira* sp. until all ponds reach the similar density of *Thalassiosira* sp. (10^5 cells/mL). Then, post larvae *Litopenaeus vannamei* (PL 7–8) were stocked into 16 semi-indoor concrete tanks (8 × 8 × 1 m) with density of 500 PL/m^2 in a completely randomized design.

There are four different treatments with four replications per treatment of Aquaritin Aqua (AA) nano nutrients that were diluted in water at recommended ratio of dilution of 1 : 1000 and applied in the culture system, namely: (1) 0,70 mg/L; (2) 0,525 mg/L, (3) 0,35 mg/L, and (4) control group or without any AA application, but included standard application of using common fertilizer (Urea and NPK fertilizers) to enhance the growth of diatom. The production period was 90 days with the addition of AA were conducted every 10 days during the culture period. The density of *Thalassiosira* sp. as the cultured diatom was measured using microscopic method one day prior to the addition of AA and one day after the addition of AA using hand-held water sprayer (5 L in capacity) (ACE hardware) that was calibrated to spray 20 mL per cycle. For the control group, the growth of diatom that was triggered by the addition of commercial organic fertilizers, the density of diatom were measured similar with the AA treatment group. Cultured tanks were filled with water with a salinity of 30–33 g/L. The primary source of mechanical aeration was with an air disc fine bubble diffuser, with one 0.5 HP paddlewheel (Minipadd™) per tank providing additional aeration. Water exchange was 5–10% throughout the 90 days trial.

Feed management

Shrimp in all the tanks were fed with the same diet (33–35% crude protein, 5% crude lipids) produced by Evergreen (Indonesia Evergreen Agriculture, Lampung Selatan) throughout the growth trial. The amount of feed used in this experiment was calculated based on the expected weight gain of 1 g/week, a feed conversion ratio (FCR) of 1.4 and a weekly mortality of 3% during the grow-out period. During the trial, shrimp were fed six times per day and the daily ration was adjusted based on the percentage of body weight after sampling the shrimp on a weekly basis.

Growth sampling and water quality

Shrimp were sampled weekly throughout the production cycle using a hand net (0.5 m in diameter and 1 cm mesh size) to collect approximately 20–30 individuals per tank. Water quality (DO, pH, temperature, and salinity) was monitored four times per day (06.00–07.00 h; 14.0–15.00 h; 17.00–18.00 h and 23.00–24.00 h) using real-time water quality sensors (Aqua Troll 500, In-Situ Inc., Fort Collins, CO, USA) and managed by AquaEasy Smart Aquaculture apps (BOSCH, Singapore). Secchi disk readings were recorded once a week. Ammonia nitrogen (NH_3-N) was analyzed with ultraviolet/visible spectrophotometer (PerkinElmer, Lambda XLS, USA) once a week (Table 1).

Meanwhile, nitrite nitrogen (NO_2-N) and total ammonia nitrogen (NH_3-N) were analyzed using HACH DR890 colorimeter (Hach Company, Love- land, CO, USA) twice a week (Table 1). At the end of the growth trial, shrimp were harvested fully, counted and batched weighed to calculate the final biomass, final weight, percentage weight gain (%WG), FCR, survival (SR), and voluntary feed intake (VFI) as shown in Table 2.

Table 1. Overall water quality measurements during the 90 days shrimp grow-out phase of the experiment. Data are presented as mean ± standard deviation (range).

Time	Parameters					
	Temperature (°C)	Dissolved oxygen (mg/L)	pH	Salinity (g/L)	Ammonia (mg/L TAN)	Nitrite (mg/L NO_2-N)
AM	27.76 ± 0.85	5.96 ± 0.23	7.81 ± 0.09	24.22 ± 2.74	0.38 ± 0.09	0.28 ± 0.04
PM	29.27 ± 0.98	5.71 ± 0.41	7.69 ± 0.26	23.60 ± 8.51		

$$\% \text{ WG} = \frac{(\text{average final weight} - \text{average initial weight})}{(\text{average initial weight})} \times 100$$

$$\text{FCR} = \frac{\text{Feed given (g)}}{\text{Alive weight gain (g)}}$$

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

Protein composition analysis

Upon termination of the trial, four shrimp from each tank or sixteen shrimp per treatment were randomly sampled and stored at -60°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 combustion according to the DUMAS Method (ISO 16634-1; ISO, 2008) and performed by the Bogor Agricultural University (Bogor, West Java, Indonesia)

Statistical analysis

All growth parameters were analyzed using one-way analysis of variance (ANOVA) to determine the significant differences among treatments followed by Tukey's multiple comparison tests to determine the difference between treatment means in each trial. All statistical analyses were conducted using SAS system (V9.4. SAS Institute, Cary, NC, USA).

RESULTS AND DISCUSSION

The present study demonstrates the effectiveness of Aquaritin Aquaculture (AA) contains with a mix of 11 nano-scale nutrients (minerals) and proprietary mineral compound called SN 25 to enhance the growth of diatom *Thalassiosira* sp. and shrimp *Litopenaeus vannamei* in the culture ponds. The better growth performance of shrimp also complemented with better feed utilization efficiency, not only to the formulated diet but also to the phytoplankton floc, especially diatom *Thalassiosira* sp. throughout the 90 days of the culture period.

Microalgae such as diatoms and green algae can grow naturally and develop in shrimp pond production system, and shrimp can get the benefit through the continuous consumption of the phytoplankton floc (Tacon *et al.*, 2002; Shaari *et al.*, 2011; Sotomayor *et al.,* 2019). Our study indicated that the addition of Aquaritin Aquaculture where the production involves sequential loading of nano-adsorbates on nano-adsorbents through a unique process that allows cations and anions to be loaded on a single formulation could effectively extent the growth of *Thalassiosira* sp.. The growth of *Thalassiosira* sp. was highest in the group treated with 0,70 mg/L, followed by 0.53 mg/L, 0.35 mg/L and the control group. Despite all groups has similar decreasing trend, but the group of 0.70 mg/L could hold the number of *Thalassiosira* sp. in the ponds compared to other treatment. During the last five weeks of observations, the density

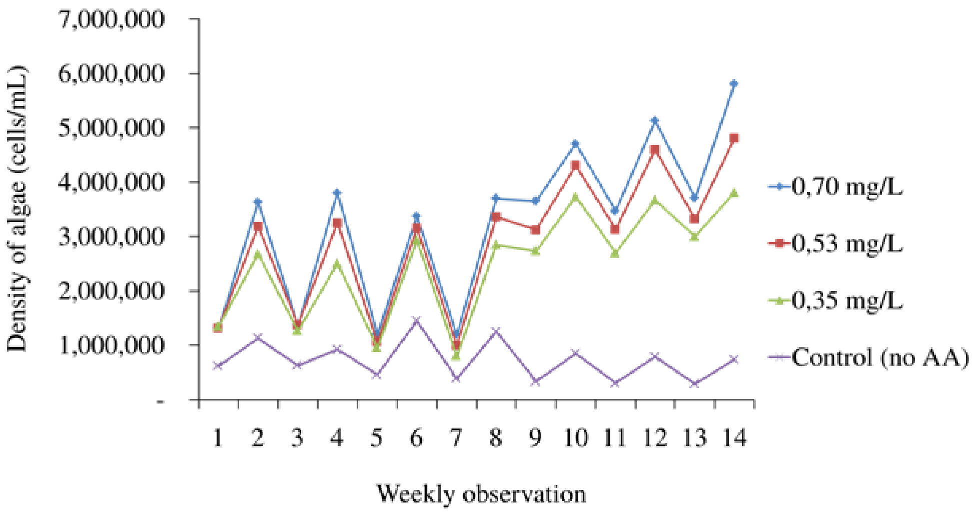

Figure 1. Cell densities profile of *Thalassiosira* sp. in the cultured tanks during the periods of before and after the addition of Aquaritin Aquaculture with 4 (four) different concentration, namely: (1) 0.70 mg/L, (2) 0.53 mg/L, (3) 0.35 mg/L and (4) control treatment

of *Thalassiosira* sp. were higher than the first four weeks of the growth trial in the concrete ponds containing shrimp. This could be due to the excess of remaining feeds, feces and also the accumulation of organic materials become the substrate to support the growth of the diatom. Looking at the lower growth of diatoms in the control treatment, this could be due to the lower fixation rate of common fertilizers added into the pond.

The stocking density used in this growth trial was 500 PL/m^2 and specified as an intensive scale of shrimp culture system (Gao *et al.,* 2012; Primphon *et al.,* 2016; Zulkarnain *et al*., 2020). In this type of culture system, applying appropriate feeding strategies are important to ensure the optimization of feed utilization, which also affect the farm productivity, FCR, growth rate, water pollution, and economic returns of the culture system (Van *et al*., 2017). For intensive culture system, feed input could be either applied at a standard ration to optimize growth and economic return or at restricted rations to reduce the FCR during the culture period. However, if we focus on economic returns, further optimizing the levels of feed inputs can be achieved by encourages the shrimp to utilize the natural foods (Jatobá *et al*., 2014; Van *et al*., 2017). Results of our feeding trial indicated that adding Aquaritin Aquaculture to enhance the growth of *Thalassiosira* sp. could also improve the biomass, final mean weight, survival, percentage weight gain and better feed utilization in terms of FCR during the culture period. Shrimp in the enhanced ponds (0,70 mg/L) had better final biomass (kg), final mean weight (g), survival (%), weight gain (%) and lowest FCR than those in the group of 0.53 mg/L, 0.35 mg/L and control treatment (Figure 3 and Table 2). Shrimp treated with 0.70 mg/L of AA had a better FCR compared to other group and this could be due to the presence of *Thalassiosira* sp. in sufficient number to support the growth and fulfill the nutrient requirement of shrimp *L. vannamei.*

Nutritional study in the past indicate that both ɷ-6 and ɷ-3 fatty acids are dietary essential for juvenile of *Litopenaeus vannamei,* with ɷ-3 fatty acids promoted faster growth than ɷ-3 (Lim *et al*., 1997). Based on fatty acid composition analysis of three diatom species commonly used in aquaculture showed that the highest content of lipid was found in *Chaetoceros gracilis,* then followed by *Thalassiosira* sp., while the lowest was in *Skeletonema costatum* (Prartono *et al*., 2013). Still from the same report, Prartono *et al*. (2013) reported that the highest fatty acids methyl esters (FAME) content found in *Thalassiosira* sp.. was methyl palmitic ($C_{16:0}$) that can be obtained through extraction process using chloroform and methyl palmitoleic ($C_{16:1}$) extracted by hexane. The uses of diatoms have been reported to be beneficial algae in shrimp ponds since they could form large floc aggregates which could be ingested by shrimp (Burford, 1997; Suita *et al.,* 2015). Study from Ju *et al*. (2009) indicated that adding the whole diatom or nanno-biomass to the control diet can significantly improve shrimp growth, survival and fatty acids contents in shrimp tails. This in line with our study and indicated that the enhanced diatoms in shrimp culture had a major role in improving the growth of the shrimp.

There were also significant differences in terms of water clarity (m) during the culture period. The secchi-disk readings showed that the clarity (Figure 2) that also illustrated the density of diatoms during the culture period was lower in 0.7 mg/L group, followed by 0.53 mg/L, 0.35 mg/L and control group. The overall mean and standard deviation of morning and afternoon pH, salinity (g/L), water temperature (°C) and dissolved oxygen (mg/L) together with ammonia (mg/L TAN) and nitrite (mg/L NO_2-N) are

Table 2. Growth performance of Pacific white shrimp *Litopenaeus vannamei* (mean initial weight 0.03–0.05 g) treated with Aquaritin Aquaculture for 90 d.

Treatment	Final biomass (kg)	Final mean weight (g)	Survival (%)	WG[1] (%)	FCR[2]
0.70 mg/L	259.00[a]	10.58[a]	76.54[a]	35.150[a]	1.35[a]
0.53 mg/L	248.50[b]	10.34[b]	75.11[a]	34.359[b]	1.41[a]
0.35 mg/L	231.50[c]	10.15[c]	71.27[b]	33.733[c]	1.51[b]
0 mg/L	218.00[d]	9.58[d]	71.11[b]	31.833[d]	1.61[c]

Note: [1]WG = Weight gain; [2]FCR= Feed conversion ratio. Values represent the mean of ten replicates. Results in the same columns with different superscript letter are significantly different (P<0.05) based on analysis of variance followed by the Tukey's multiple comparison test.

displayed in Table 1. Based on the data, all the physical parameters are still within the acceptable range for *L. vannamei*. In addition, ammonia in the range of 0.38 ± 0.09 mg TAN/L and Nitrite in the range of 0.28 ± 0.04 mg/L NO_2-N also still within the acceptable range for Pacific white shrimp *L. vannamei* (Xu *et al.*, 2013)

This data also indicated that the addition of AA do not trigger the nutrient-rich water condition within the culture system. The application of treatment ponds in this study that include the hydraulic retention times in combination with biofiltration process could also effectively minimize the possibility of serious eutrophication in the surrounding water environments. High turbid waters, as indicated by the low secchi-disk reading, are likely due to the growth of *Thalassiosira* sp. in the culture ponds. With respect to the present study, growth rates of *Thalassiosira* sp. increased in response to the increasing addition level of AA into the culture environment. The use of hand-held water sprayer in this study may help to speed-up the distribution process of nano-protein and enhanced the penetration of AA to the culture environment. This study further explains due to the high reactivity of nano-nutrients, resulting in an increase and effective absorption of nutritional elements to support the growth of *Thalassiosira* sp.

The addition of AA to enhance the growth of *Thalassiosira* sp. also provides a beneficial

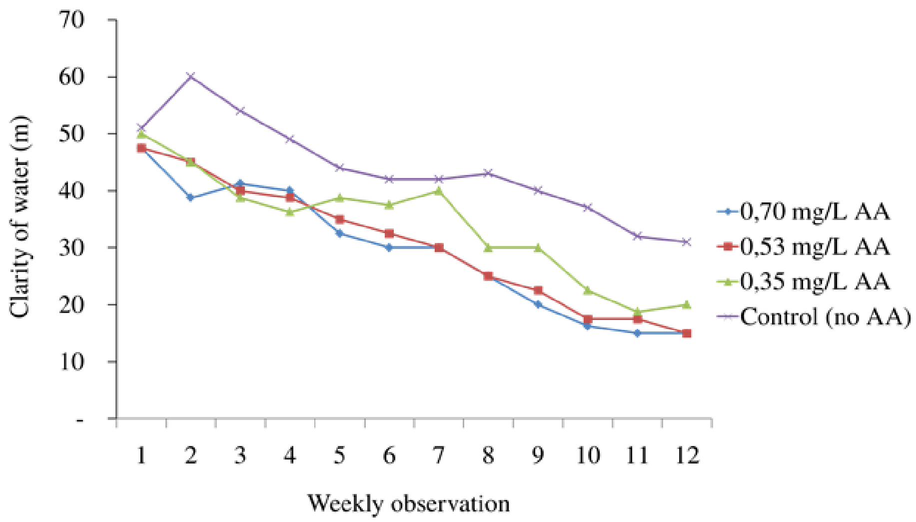

Figure 2. Profile of the clarity of water during the culture of the shrimp *L. vannamei* enriched with three different concentrations of Aquaritin Aquaculture and control treatment.

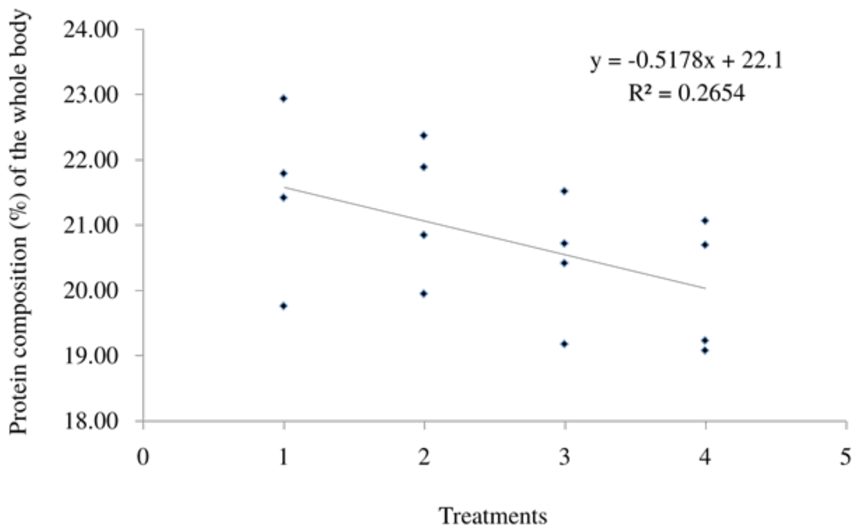

Figure 3. Protein composition (%) in the whole body of shrimp treated with (1) 0.70 mg/L, (2) 0.53 mg/L, (3) 0.35 mg/L Aquaritin Aquaculture and (4) control treatment. Values represent the mean of four replicates.

impact to the protein composition in whole body of shrimp (Figure 3). Despite statistically, there is no significant differences were observed in the protein level in the whole-body of shrimp across all treatments. Biologically, the protein composition in the whole body of shrimp treated with 0,70 mg/L was higher compared to the group of shrimp treated with 0,53 mg/L, 0,35 mg/L and control group. The results of this research indicate that the use of AA to enhance the growth of *Thalassiosira* sp. may led to an adequate nutritional availability to fulfill the specific nutrient requirement of *L. vannamei.*

CONCLUSION

The findings from this study can be summarized in a conceptual model where the addition of Aquaritin Aquaculture (AA) could enhanced the growth of *Thalassiosira* sp. as the good source of fatty acid to fulfill the specific nutrient requirements of shrimp. The utilization of natural phytoplankton floc by the shrimp during the culture period can improve the growth rates and nutritional profile of the Pacific white shrimp *L. vannamei*. The better growth performance indicates the potential advantages of using Aquaritin Aquaculture as an effective nutrient source to develop the phytoplankton flocs in shrimp ponds and support the growth and nutritional profile of shrimp during the culture periods. In future studies, we can analyze the nutritional effects of AA to enhance the growth of other microalgae and also the growth and fatty acid composition of *L. vannamei.*

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