Growth performance of white shrimp *Litopenaeus vannamei* fed with Various dosages of prebiotic honey

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Abstract. The intensive culture system of white shrimp with high stocking density leads to slow shrimp growth performance. An alternative way to increase the growth performance of white shrimp is using a prebiotic. This study was aimed to evaluate the effectiveness of honey as a prebiotic through commercial feed to improve the growth performance of white shrimp. The white shrimp were reared with comercial feed that have been enriched with honey. This study consisted of four treatments, which were: control (without prebiotic), and the giving of honey at dosages of (A) 0.2%; (B) 0.4%; (C) 0.6%, each treatment with three replications. The average weight of vaname shrimp used was 0.44±0.11 g, maintained with a density of 270 shrimp/m^{-3} with an aquarium size of (60×30×30 cm^3). The results of this study indicate that honey with a dosage of 0.6% results in the best growth performance with a growth rate of 0.14±0.002 g/day, specific growth rate of 4.70±0.02%/day, and feed conversion ratio of 1.53±0.02.

Keywords: growth rate, honey, prebiotic, white shrimp

1. Introduction

One of the fastest growing species with a high market demand is white shrimp *Litopenaeus vannamei.* Several countries that import white shrimp are the European Union (547,000 tons), China (300,000 tons), the United States of America (268,000 tons), and Japan (163,545 tons) [1]. White shrimp are an introduced species that grows enormously in Indonesia. The culture activity of white shrimp started in 2001, right after the production of tiger shrimp *Penaeus monodon* started to decline [2]. White shrimp have several excellences, such as faster growth (around 3.5 g/weeks, while tiger shrimp growth is around 3 g/week), advanced immune system, capabilty to be cultured in high density (150 ind.m^{-3}), high tolerance to salinity (0.5−45 ppt), and better feed conversion rate (1.2−1.6) [3]. High market demand requires high production using intensive aquaculture in order to achieve massive production briefly [4].

Intensive aquaculture application in white shrimp production is conducted using a high stock density. It triggers several obstacles, such as stress condition, water quality depression, and low feed efficiency. Moreover, intensive shrimp culture has higher possibility of disease outbreak bacause of the unstable condition [5]. The common method to overcome disease outbreak in white shrimp culture is antibiotic application. However, antibiotics application in white shrimp culture potentially causes bacteria resistance and antibiotics accumulation in shrimp [6]. Therefore, it is necessary to invent another solution to increase the growth rate and prevent a

disease outbreak without gaining negative side effects. One of the solutions is prebiotic utilization.

Prebiotics are oligosaccharide compounds, such as fructooligosaccharides (FOS), mannanoligosaccharides (MOS), and galactooligosaccharides (GOS) which are unable to be digested by the host, but induces the normal microflora to grow in the digestion tract [7]. A food material is catagorized as prebiotics if it complies to the following criteria, such as resistance to gastric acid, fermentable by advantageous bacteria, and the fermented product affects the host's immune system [8]. Prebiotics have been proven to boost growth rate, feed efficiency, digestbility, and survival rate on some aquaculture species [9, 10]. Prebiotics act as substrates for advantageous bacteria to grow in the colon. The advantageous bacteria produce enzymes to assist in the digestion process [11]. Moreover, prebiotics utilization also induces the immune system. Prebiotics stimulate advantageous bacteria to grow selectively. Those bacteria are able to interact with gut associated tissue (GALT) which accelerates the proliferation of the immune cell [12]. A FOS utilization for six weeks is able to increase the non-specific immune system in white shrimp [13].

One source which has the potential to act as a prebiotic is honey. The honey contains are 17.2% water, 38.2% fructose, 31.3% glucose, 0.7% sucrose, and 3.1% oligosaccharide [14]. Oligosaccharide is a form of component which is unable to be hidrolized or absorbed by the small intestine, but it is fermentable in the colon and induces advantageous bacteria growth [15]. Honey is a potential source of prebiotic because it can increase the population of advantageous bacteria (*Bifidobacteria* and *Lactobacilli*) in the digestion tract when it was tested in vitro [16]. However, it is essential to further study the utilization of honey as a prebiotic in white shrimp culture. This study was aimed to evaluate the effectivness of honey as a prebiotic source to aid in increasing the growth performance of white shrimp *L. vannamei* which is delivered through commercial feed.

2. Materials and Methods

2.1. Experimental preparation

This study used 15 aquariums with 60×30×30 cm^3 in size. Those aquariums were cleaned up and then dried. After they were completely dry, the aquariums were disinfected using chlorine (30 mg/L), aerated, and left for 24 hours. After disinfection, the aquariums were rinsed using freshwater and then filled with 27 L of seawater. Aeration and top filter were set on each aquarium to maintain water quality and oxygen supply.

2.2. Shrimp preparation

The experimental shrimp for this study were post larvae 10 (PL 10) from PT. Suri Tani Pemuka, Carita Unit, Pandeglang, Banten. Before the treatment was started, the post larvae were reared for 30 days using a fiber tank (2×1×0.5 m^3) filled with 200 L of water. The post larvae were fed using artemia for the first 14 days, continued using commercial feed until day 30 to achieve desired size. After the rearing activity was done, the shrimp were fasted for 24 hours to reduce the remaining feed in the digestion tract. The shrimps were sampled using 30 ind to measure its growth and the result was an average growth of 0.40±0.11 g. Moreover, as many as 15 shrimps were put into the aquarium.

2.3. Feed preparation

The experimental feed was pellet with 40% protein content. It was combined with honey from bee farmers in Depok, West Java. The enrichment was done using a spray method. The commercial feed was added with 0%, 0.2%, 0.4%, and 0.6% (v/w) of honey concentration, depending on the treatment. The enrichment process was started by adding egg white (albumin) to the feed. The honey was diluted using PBS (*phosphate buffered saline*) with a ratio of 1:1, complied on the treatment. After that, as much as 2% (v/w) of egg white was added to the feed

as a binder. The prebiotics and the remaining egg white were mixed, and sprayed on to the feed. After the feed was completely sprayed by the mixture, it was wind-dried and put in a container.

2.4. Shrimp rearing

The white shrimp was reared for 70 days and fed using experimental feed based on the treatment. The feeding frequency was five times a day (at 06.00, 10.00, 14.00, 18.00, and 22.00). The feeding method used was satiation. The rearing media was maintained by siphoning 10% of total volume everyday, water discharging of 50% every two days, and a circulation system using top filter. The water quality parameters during the study were 5.0–6.2 mg L^{-1} of dissolved oxygen, 27.9–29.3ºC of temperature, 7.0–7.3 of pH, 0.00–0.10 mg L^{-1} of total ammonia nitrogen, 0.00–0.20 mg L^{-1} of nitrite, 0.24–1.04 mg L^{-1} of nitrate, and 26–33 g L^{-1} of salinity.

2.5. Sample collection

The weight of the white shrimp was measured every two weeks. As many as 15 shrimps were collected and put into a container filled with seawater. Furthermore, the shrimps were weighed using a digital scale (accuration 0.01 g), then the sample was returned to the container.

2.6. Experimental design

The experimental design used a completely randomized design, consisting of four treatments and three replications as shown in table 1. This study was designed using an experimental method, the results were tabulated using Microsoft Excel 2010.

Table1. Experimental design of prebiotics honey towards white shrimp through artificial feed.

Treatment	Description
Control	Artificial feed, no prebiotic
A	Artificial feed, 0.2% of prebiotic honey
B	Artificial feed, 0.4% of prebiotic honey
C	Artificial feed, 0.6% of prebiotic honey

2.7. Experimental parameters

2.7.1. Survival rate. Survival rate is the ratio between final population towards initial population. It was calculated using the following formula [17]:

$$SR = \frac{Nt}{No} \times 100 \quad (1)$$

Notes :
SR = Survival rate (%)
Nt = Final population (individual)
No = Initial population (individual)

2.7.2. Daily Growth. Daily growth is the average weight increase from the beginning until the of the study. It was calculated using the formula below [18]:

$$\text{Daily growth} = \frac{Wt - Wo}{t} \quad (2)$$

Notes :
Wt = Final average weight (g)
Wo = Initial average weight (g)
t = Rearing period (day)

2.7.3. Specific growth rate. Specific growth rate is the daily weight addition percentage during the rearing period. The parameter was calculated using the following formula [19]:

$$SGR = \left[\sqrt[t]{\frac{Wt}{Wo}} - 1\right] \times 100 \quad (3)$$

Notes :
SGR = Specific growth rate (%/day)
Wt = Final average weight (g)
Wo = Initial average weight (g)
t = Rearing period (day)

2.7.4. Feed consumption. Feed consumption amount was determined from the spread between feed amount before being given to the shrimp and the remaining feed [17].

2.7.5. Feed conversion ratio. Feed conversion ratio(FCR) is a unit to desribe a certain feed amount to earn one kilogram of biomass. FCR was calculated using the formula below [20]:

$$FCR = \frac{F}{[(Bt+Bm)-Bo]} \quad (4)$$

Notes :
FCR = Feed conversion ratio
F = Total feed amount (g)
Bt = Final biomassa (g)
Bm = Deceased biomass (g)
Bo = Initial biomass (g)

2.7.6. The abundance of intestine bacteria. The abundance of bacteria in the shrimp intestine was calculated before and after treatment using the total plate count method [21]. As much as 0.1 g of shrimp intestine was weighed, crushed, and added to 0.9 mL of PBS. Furthermore, it was put in a sterile tube and homogenized using vortex. As much as 0.1 mL from the mixture was conducted serial diluted for seven times, then 0.05 mL from the result of serial dilution was collected using a micropipet and evenly spread on a sea water complete (SWC) media and incubated for 48 hours. The bacteria colony was counted and mutiplied by the dilution factor.

$$\text{Total bacteria} = \text{the number of colony} \times \frac{1}{FP} \times \frac{1}{Sample\ volume} \quad (5)$$

Notes :
The number of colony = The number of colony (log CFU/g intestine)
FP = Dilution factor (10^{-n})

2.8. Data analysis
All of the data of experimental parameter was tabulated using Microsoft Excel 2010 and analyzed using analysis of variance (ANOVA) in SPSS 16.0 in confidence level of 95%. The significant difference would be analyzed further using a Duncan test. A descriptive analysis was conducted to calculate bacteria abundance and water quality.

3. Results and Discussions

3.1. Results
3.1.1. Survival rate. The survival rate of the white shrimp after 70-day of rearing using honey as a prebiotic with various dosages is shown in figure 1. There was no significant difference between treatments (P>0.05). The survival rate of treatment A was 88±3.85%, while the treatment B and C were 91±3.85 %.

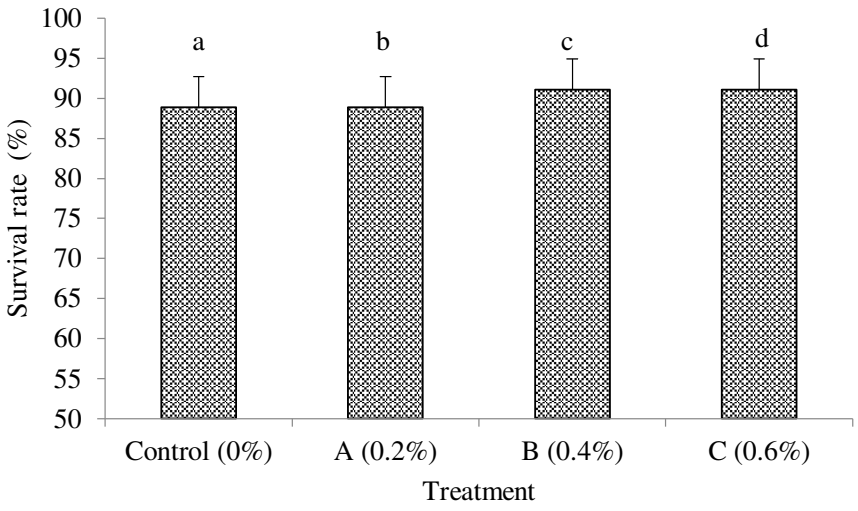

Figure 1. Survival rate of the white shrimp with honey as a prebiotic with various dosages after 70 days of rearing. The same letter in the graphic indicates no significant difference between treatment (Duncan P>0.05).

3.1.2. Average weight. The average weight of the white shrimp after 70 days of rearing is shown in figure 2. The average weight was consistently increasing until the end of the study. The initial weight was 0.40±0.11 g and at the end of the study, it reached up to 7.71±0.14 −10.00±0.11 g.

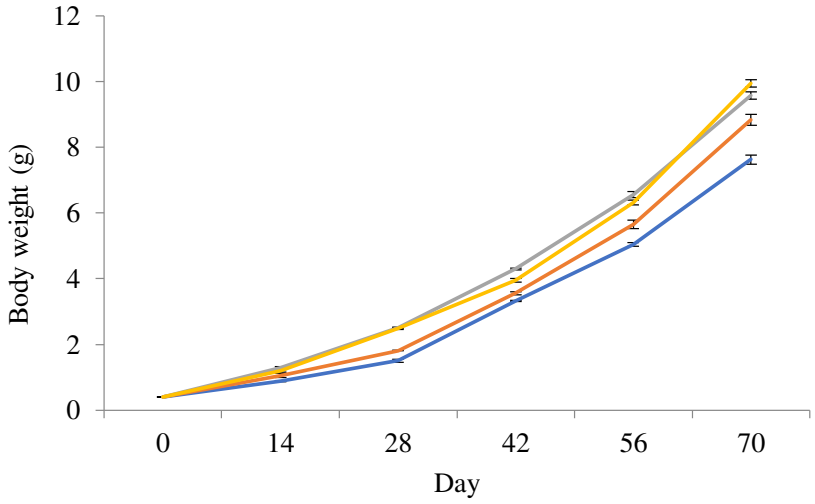

Figure 2. Average weight of the white shrimp after 70 days of rearing fed using prebiotic honey at various dosages, — control (0%); — A (0.2%); — B (0.4%); — B (0.6%).

3.1.3. Growth rate. The daily growth rate of the vannamei shrimp after 70 days of rearing is shown in figure 3. The daily growth rate in all of the treatments was significantly different (P<0.05). The highest daily growth rate was obtained in treatment C (0.14±0.002 g/day), followed by treatment B and A in with 0.13±0.002 g/day and 0.12±0.002 g/day, respectively, meanwhile the lowest daily growth rate was in control treatment (0.10±0.002 g/day).

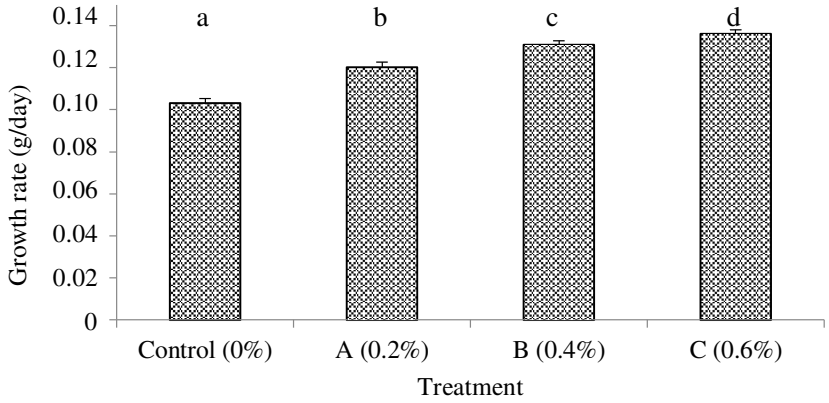

Figure 3. The daily growth rate of the vannamei shrimp with honey as prebiotic after 70 days of rearing. The different superscript letters in the chart show significant differences within treatments (Duncan P<0.05).

3.1.4. Spesific growth rate. The specific growth rates of the vannamei shrimp after 70 days of rearing are shown in figure 4. The specific growth rates in all of the treatments were significantly different (P<0.05). The best specific growth rate was obtained in treatment C (4.70±0.02%/day), followed by treatment B (4.64±0.02%/day), treatment A (4.52±0.03%/day), and the lowest was obtained in control treatment (4.30±0.03%/day)

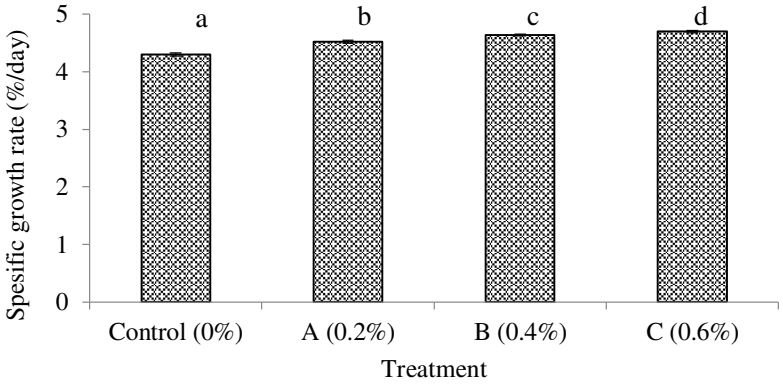

Figure 4. The specific growth rate of vannamei shrimp with honey as prebiotic after 70 days of rearing. The different superscript letters in the chart show significant differences within treatments (Duncan P<0.05).

3.1.5. The Total amount of consumed feed. The total amounts of consumed feed with the addition of honey as a prebiotic after 70 days of rearing are shown in figure 5. The total amounts of consumed feed in all of the treatments were significantly different (P<0.05). The highest amount was in treatment B (241±10g), followed by treatment C (218±8 g) and treatment A (191±1 g), meanwhile the lowest amount was in control treatment (176±5g).

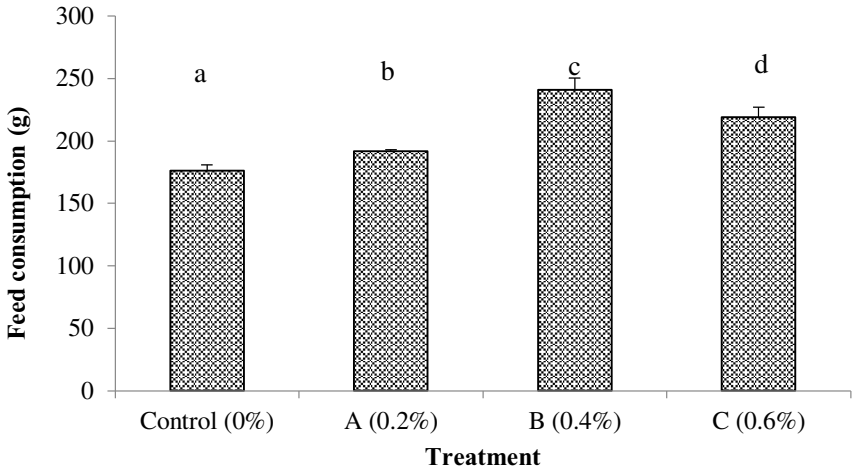

Figure 5. The total amount of consumed feed of vannamei shrimp with honey as prebiotic after 70 days of rearing. The different superscript letters in the chart show significant differences among treatments (Duncan P<0.05).

3.1.6. Feed conversion ratio. Feed conversion ratios with the addition of honey as a prebiotic in the vannamei shrimp after 70 days of rearing are shown in figure 6. Feed conversion ratios among prebiotic treatments were significantly different with the control (P<0.05). The best feed conversion ratio was treatment C (1.53±0.02).

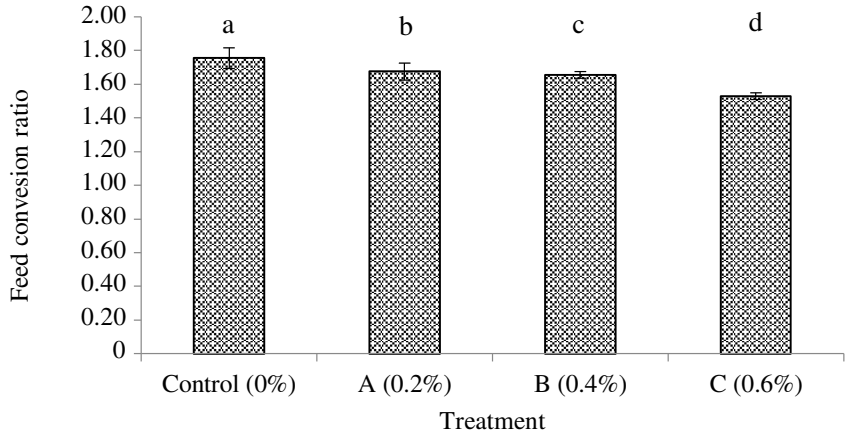

Figure 6. The feed conversion ratio of vannamei shrimp with honey as prebiotic after 70 days of rearing. The different superscript letters in the chart show significant differences among treatments (Duncan P<0.05).

3.1.7. The Abundance of intestine bacteria. The total amounts of bacteria in vannamei shrimp's intestine after 70 days of rearing fed with addition of honey are shown in figure 7. The abundances of bacteria in the intestine in all treatments were significantly different (P<0.05). The highest result was obtained in treatment C (9.73±0.04 log CFU/g).

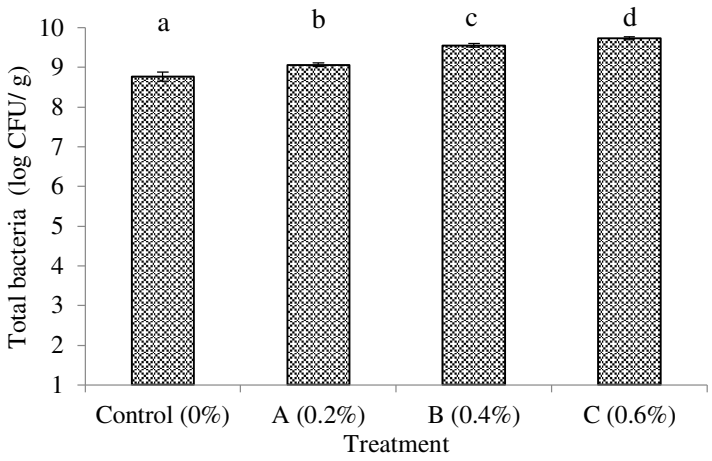

Figure 7. The total amount of bacteria in vannamei shrimp's intestine fed with honey as prebiotic after 70 days of rearing. The different superscript letters in the chart show significant differences among treatments (Duncan $P<0.05$).

3.2. Discussion

The survival rate is a percentage of surviving fish from the start of rearing until the end of the rearing [17]. The survival rates of the vannamei shrimp after 70 days of rearing were not significantly different among all treatments, ranging between 88%–91%; in line with the survival rate of *totoaba fish* (*Totoaba macdonaldi*) fed with GroBiotic®-A [22] prebiotic which was not significantly different among all treatments. The result was also in line with the turbot (*Psetta maxima*) rearing which were fed with rafinose [23]. It was suspected that either shrimp fed with the control or the prebiotic treatments were in a controlled environment and healthy condition. Instead, honey as a prebiotic in feed has no negative effect on vannamei shrimp.

The daily growth rates and the specific growth rate in the vannamei shrimp after 70 days of rearing in all treatments were significantly different in all treatments. The highest growth rate was in treatment C (0.14 ± 0.002 g/day) and the highest specific growth was in treatment C (4.70 ± 0.02%/day), therefore to obtain 14 g as a harvest size would take 100 days. The average of the daily growth rates of the vannamei shrimp was 0.12 g/day with 116 days of harvest time. A previous study [24] shows that the addition of scFOS (*short-chain fructooligosaccharides*) as a prebiotic could increase the daily growth rate and the specific growth rate of vannamei shrimp.

The total consumed feed and feed conversion ratio of vannamei shrimp after 70 days of rearing with honey as a prebiotic in different doses showed a positive response. The highest total consumed feed was in treatment B (241 ± 10 g), whereas the lowest was in control treatment (176 ± 5 g). The total consumed feed in treatment C was lower than the treatment B (218 ± 8 g). It shows that as the prebiotic concentration increases, the daily total consumed feed decreases [25].

The value of feed conversion ratio in all treatments was significantly different than the control. The best feed conversion ratio was in treatment C (1.53 ± 0.02). The feed conversion ratio is the main parameter to figure out the feed efficiency. Feed conversion ratio is total feed ratio to produce 1 kg of shrimp [26]. As the feed conversion ratio gets lower, the efficiency of feeding becomes higher.

The addition of honey as a prebiotic can increase feed efficiency. This increase is suspected to be due to the ability of the prebiotic to increase advantageous bacteria population in the digestive tract. The addition of probiotic extracted from sweet potato helps to increase the digestibility and feed efficiency [27] while the use of MOS as a prebiotic also improves the

intestine morphology through increasing the density of the intestine microphili, hence the utilization of feed becomes more effective [28, 29].

Honey that works as a prebiotic is proven by the total bacteria in intestines. The total bacteria after the addition of honey in all treatments showed significantly different results. The addition of honey could increase the total bacteria in intestines. The highest total bacteria was in treatment C (9.73±0.04 log CFU/g). This finding was in line with the addition of honey that is proven to elevate the population of *Lactobacillus acidophilus* and *Lactobacillus plantarum* bacteria in rat's intestines [30]. The addition of honey also increases the population of lactic acid bacteria (BAL), therefore the growth of pathogenic bacteria could be supressed [31].

The increase of microflora in intestines is affected by the addition of probiotic that is fermented in the colon, resulting in a short chain fatty acid (SCFA) that consists of acetat, propionate, lactic, and butyrate acid [32]. SCFA is utilized by the advantageous bacteria in the colon therefore the population of the advantageous bacteria becomes dominant [33]. Besides that, the SCFA is also absorbed by the epithelial cells in intestines to provide the entherocyte cells to absorb the nutrient [31]. The abundance of bacteria in the digestive tract of aquatic organisms is affected by feed intake, environmental condition, nutrient, feed absorption, protein digestion, and digestion enzymes [34].

Honey as a prebiotic is also known to have a hidroscopic characteristic that causes the pathogenic bacteria to experience dehydration [35]. Honey can work as an antimicrobial agent because it contains hydrogen peroxide, as the higher the honey dose, the bigger its utility as an antimicrobial agent [36]. Honey can also trigger the growth of advantageous bacteria to produce the bacteriocin in order to inhibit the growth of pathogenic bacteria.

4. Conclusion

The addition of honey as a prebiotic can increase the growth performance of vannamei shrimp including the daily growth rate, the specific growth rate, and decrease the feed conversion ratio. The best dosage of honey as a prebiotic was obtained at 0.6%.

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