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GROWTH PERFORMANCES AND INTESTINAL BACTERIAL POPULATIONS OF PACIFIC WHITE SHRIMP (*Penaeus vannamei*) FED WITH DIFFERENT DIETARY PREBIOTICS-SUPPLEMENTED FEED

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ABSTRACT

Prebiotic applications in aquaculture are mainly given in the form of single or mixed prebiotics. A number of studies compared the effects of different doses or frequencies of a single prebiotic application. However, studies comparing different prebiotics in order to find the most effective ones for certain farmed species are limited. This study aimed to evaluate the effects of different dietary prebiotics on the growth performances and intestinal bacterial populations of Pacific white shrimp (*Penaeus vannamei*). Four treatments with triplicates were arranged in a completely randomized design (CRD). The treatments consisted of feed supplemented with different dietary prebiotics for Pacific white shrimp, including control (without dietary prebiotic), 0.5% honey (v/w), 0.5% mannan-oligosaccharide (MOS) (w/w), and 0.5% inulin (w/w). Pacific white shrimp (1.59 ± 0.12 g) were randomly stocked in 12 glass tanks (60 x 30 x 40 cm^3) with a stocking density of 15 shrimp per tank. The shrimp were fed the experimental feed to apparent satiation four times daily for 30 days. Growth parameters observed consisted of final weight, specific growth rate (SGR), feed conversion ratio (FCR), survival of Pacific white shrimp, total bacterial count, total *Vibrio* count, and dominance of *Vibrio* in the intestine of experimental shrimp. Dietary prebiotics improve the growth performances of Pacific white shrimp. The highest growth performances were found in the shrimp treated with dietary honey. The improvement in growth performance may be due to the ability of honey to boost the proliferation of beneficial bacteria in the intestines of Pacific white shrimp.

KEYWORDS: growth; honey; inulin; MOS; Pacific white shrimp; prebiotics

ABSTRAK: Kinerja Pertumbuhan dan Populasi Bakteri Usus Udang Vaname (*Penaeus vannamei*) dengan Pemberian Pakan Prebiotik Berbeda

Pemanfaatan prebiotik dibagi menjadi dua kelompok yang terdiri atas prebiotik tunggal dan prebiotik campuran. Banyak penelitian sebelumnya yang berfokus pada perbandingan dosis atau frekuensi satu jenis prebiotik tetapi tidak membandingkan jenis prebiotik yang berbeda untuk menemukan prebiotik yang paling efektif untuk spesies tertentu. Penelitian ini

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*bertujuan untuk mengevaluasi pengaruh pemberian pakan prebiotik yang berbeda terhadap kinerja pertumbuhan dan populasi bakteri usus udang vaname (**Penaeus vannamei**). Penelitian ini dilakukan melalui rancangan acak lengkap (RAL) dengan empat perlakuan dan tiga ulangan. Perlakuan yang diberikan dalam penelitian ini terdiri atas pemberian pakan prebiotik yang berbeda pada udang vaname meliputi kontrol (tanpa prebiotik), madu 0,5% (v/b), mannan-oligosakarida (MOS) 0,5% (b/b), dan inulin 0,5% (b/b). Udang vaname (1,59 ± 0,12 g) ditebar secara acak dalam 12 akuarium kaca (60 x 30 x 40 cm^3) dengan padat tebar 15 udang per akuarium. Udang diberi pakan percobaan sampai kenyang empat kali sehari selama 30 hari. Parameter yang diamati terdiri atas bobot akhir, laju pertumbuhan spesifik (LPS), rasio konversi pakan (RKP), kelangsungan hidup udang vaname, jumlah bakteri total, jumlah **Vibrio** total, dan dominasi **Vibrio** dalam usus udang percobaan. Pemberian pakan prebiotik meningkatkan kinerja pertumbuhan udang vaname. Kinerja pertumbuhan tertinggi ditemukan pada udang yang diberi madu. Peningkatan kinerja pertumbuhan ini mungkin disebabkan oleh kemampuan madu dalam meningkatkan perkembangbiakan bakteri menguntungkan di usus udang vaname.*

KATA KUNCI: inulin; madu; MOS; pertumbuhan; prebiotik; udang vaname

INTRODUCTION

Shrimp farming is one of the highly profitable aquaculture industries due to the rising global demand for shrimp products (Cornejo-Granados *et al.*, 2018). Currently, Pacific white shrimp (*Penaeus vannamei*) is the most dominant shrimp species cultivated globally to supply the demand (Zhou *et al.*, 2020). Indonesia sits in the top five producers of Pacific white shrimp worldwide, along with Ecuador, China, India, and Vietnam, contributing 74% of global production in 2023 (Jory, 2023). The intense global market competition has led to several policy and program priority changes in the Indonesian government aimed at improving national shrimp production. Unfortunately, several issues, such as poor water quality and disease outbreaks, remain the main challenges to increasing shrimp production, including feed efficiency (Cornejo-Granados *et al.*, 2017). These challenges could be prevented with adequate management in shrimp farming, such as routine pond management and modification of shrimp's microbiomes, both of which support the effort to reduce the use of antibiotics in shrimp farming (Bossier *et al.*, 2016; Ochoa-Romo *et al.*, 2022; Stentiford *et al.*, 2017).

The modification of an animal's microbiota can be facilitated by applying probiotics and prebiotics (Mohan *et al.*, 2017; Vadstein *et al.*, 2013). Prebiotics are non-digestible ingredients selectively utilized by host microbiota, conferring a health benefit (Gibson *et al.*, 2017). There are several prebiotics that have been commonly used in aquaculture, including fructooligosaccharides (FOS), galacto-oligosaccharides (GOS), mannan-oligosaccharides (MOS), inulin, and β-glucanase (Song *et al.*, 2014). Prebiotics are proven to improve intestinal microvilli, increase immune response, and enhance the growth performance of cultured biota (Sang *et al.*, 2014). Moreover, prebiotics can prevent the attachment of pathogens to epithelial cells, eliminate pathogens from gut epithelial cells by acting as receptors and immune system modulators, and play a role as inflammation controllers (Al-Sheraji *et al.*, 2013; Mohammadi *et al.*, 2022; Schell *et al.*, 2022).

Prebiotic applications in aquaculture are mainly in form of single or mixed prebiotics. Several single probiotics such as β-glucan, inulin, arabinoxylanoligosaccharide (AXOS), MOS, GOS, FOS, and other oligosaccharides were reported to promote growth performance, increase feed utilization efficiency, improve immune response, and increase the production of aquaculture biota (Abdel-Latif *et al.*, 2022; Li *et al.*, 2018; Li *et al.*, 2019; Li *et al.*, 2021; Shoaei *et al.*, 2015; Yilmaz *et al.*, 2022). Prebiotic mixtures also effectively improve the growth of aquaculture organisms (Wee *et al.*, 2024). Immunogen, a commercial prebiotic mixture, was proven to promote the growth of common carp (*Cyprinus carpio*) (Ebrahimi *et al.*, 2012). Honey that contains inulin, FOS, and GOS has been proven to improve growth performances and disease resistance of Pacific white shrimp (Fuandila *et al.*, 2020; Widanarni *et al.*, 2019a; Widanarni *et al.*, 2020).

The intestine is one of the most important organs in shrimp in relation to nutrient absorption and growth (Ochoa-Romo *et al.*, 2022). The host's health is also strongly affected by the structural integrity of the intestine, the immune molecules, and the intestinal microbiota (Levy *et al.*, 2017). Many reports conclude that a healthy intestinal microbiota can produce short-chain fatty acids (SCFAs) to boost the host's health (Koh *et al.*, 2016). Therefore, finding a new approach to modulate shrimp microbiota through the shrimp diet is necessary. The supplementation of prebiotics has been reported to promote growth, survival, and immune response, as well as decrease the number of pathogens in the host body organs (Duan *et al.*, 2019; Hu *et al.*, 2019). A number of studies compared the effects of different doses or frequencies of a single prebiotic application. However, studies comparing different prebiotics in order to find the most effective ones for certain farmed species are limited. This study aimed to evaluate the effects of different dietary prebiotics on the growth performances and intestinal bacterial populations of Pacific white shrimp.

MATERIALS AND METHODS

Experimental Design

The experimental units consisted of four treatments, each triplicated and arranged in a completely randomized design (CRD). The treatments consisted of different dietary prebiotics for Pacific white shrimp, including the control (without dietary prebiotic), 0.5% honey (v/w), 0.5% mannan-oligosaccharide (MOS) (w/w), and 0.5% inulin (w/w).

Experimental Animals and Containers

The experimental animals used were Pacific white shrimp (post-larvae 10) obtained from a hatchery owned by PT. Suri Tani Pemuka, Jembrana, Bali. The shrimp were acclimatized into the controlled environment using a fiber container (2 x 1 x 0.5 m^3) with a water volume of 800 L. The acclimatization was performed for 30 days prior to the feeding trial experiment. The container had top filters to apply a recirculating system to the rearing media. The shrimp were fed a commercial feed with a protein content of 35% four times daily (07:30, 12:00, 16:00, and 20:00) to apparent satiation.

Preparation of Experimental Feed

The feed used in this study was a commercial shrimp feed with a protein content of 35% (Evergreen Feed 922-0 Series) manufactured by Fujian Evergreen Feed Co., Ltd. The experimental feed consisted of the control feed and the prebiotic-supplemented feed. The prebiotics used in this study were forest honey obtained from the wild bees in the forest of Sumbawa, Nusa Tenggara Barat, MOS (Bio-MOS) obtained from Alltech Inc., KY US, and inulin (Orafti®GR) produced by BENEO-Orafti, Belgium. The control feed was prepared by mixing 2% egg white and physiological solution with a portion of 1:1 (v/v), while the prebiotic supplemented feed was prepared by mixing each prebiotic and physiological solution with a portion of 1:1 (v/v), followed by mixing each mixture with 2% egg white. Each mixture was added to the feed through spraying with doses applied in each treatment. The experimental feed was air-dried and stored in sealed plastic bags before use.

Feeding Trials

Pacific white shrimp with an average weight of 1.59 ± 0.12 g were randomly stocked in 12 glass tanks (60 x 30 x 40 cm^3) with a working volume of 60 L. The shrimp were stocked in the experimental tanks with a stocking density of 15 individuals per tank. Each tank was equipped with a top filter and a water heater to apply a recirculating system and maintain the water temperature in the rearing media.

The shrimp were fed the experimental feed to apparent satiation four times a day (07:00, 11:00, 15:00, and 23:00). The feeding trial was conducted for 30 days. The water quality of the rearing media was maintained by removing the shrimp feces and the unconsumed feed every morning and late afternoon. The water replacement of the rearing media was also performed at a volume of 20% of total water volume every 3 days. The water quality of the rearing media during the experiment was in the normal ranges for the culture of Pacific white shrimp, including temperature at 28.4-30.5°C, pH at 7.8-8.8, dissolved oxygen at 6.5-7.2 mg L^{-1}, ammonia at 0.01-0.05 mg L^{-1}, nitrite at 0.01-0.05 mg L^{-1}, nitrate at 0.01-0.05 mg L^{-1}, and alkalinity at 150-185 mg L^{-1}.

Measurement of Growth Performances

The measurement of growth performances was done through routine growth sampling (every 10 days) to record the average body weight of the shrimp and daily monitoring of shrimp survival. The growth performance parameters observed consisted of final weight, specific growth rate (SGR), feed conversion ratio (FCR), and survival of Pacific white shrimp.

Examination of Intestinal Bacterial Populations

The intestinal bacterial populations of the tested shrimp were expressed by total bacterial count, total *Vibrio* count, and dominance of *Vibrio* in the shrimp intestines. The intestines were collected from the Pacific white shrimp in each treatment at a weight of 0.1 g per sample. Total bacterial count was obtained through the total plate count method using trypticase soy agar + 1.5% NaCl (TSA + 1.5% NaCl), while total *Vibrio* count was enumerated through the total plate count method using thiosulphate citrate bile salt sucrose agar (TCBS agar). The intestinal bacterial population was examined at the end of the experiment. The use of shrimp as the animal test subject in this study follows the national standard on the use of animals in research and academics prescribed by the National Standardization Agency (2015) in SNI 2332.3:2015.

Data Analysis

The data obtained were tabulated using Microsoft Excel 2019. The normality test was performed through Shapiro-Wilk's test, while the homogeneity of variance test was done through Levene's test. The data were analyzed through analysis of variance (ANOVA) followed by Duncan's test at a confidence level of 95% using SPSS Version 27.

RESULTS AND DISCUSSION

Growth Performances

The application of prebiotics-supplemented feed resulted in higher growth than those of control. The positive contribution of dietary prebiotics on the growth of Pacific white shrimp was expressed by higher final weight and SGR on Pacific white shrimp supplemented with prebiotics compared to those of the control (6.53 ± 0.11 g; 4.71 ± 0.05% day^{-1}). Pacific white shrimp supplemented with honey showed the highest final weight (11.47 ± 0.10 g) than those of other treatments ($P<0.05$) (Figure 1). Supplementation of honey into the shrimp diet also resulted in the highest SGR (6.59 ± 0.03% day^{-1}) compared to those of other treatments ($P<0.05$) (Figure 2).

Prebiotics may improve growth rate and immune system as well as modify the bacterial community in the gastrointestinal tract of the treated biota. The important factors that should be considered to optimize the role of prebiotics as feed additives include the type of prebiotic, species, age, the production stage of the biota, the type of diet, and practical formulations (Yousefian & Amiri, 2009). It can be stated that different prebiotics may have different effects on the cultured species. The present study showed that dietary prebiotics improved the growth of Pacific white shrimp. Dietary supplementation of MOS in the feed increases the growth performance of the Pacific white shrimp (Prastiti *et al.*, 2018). It is

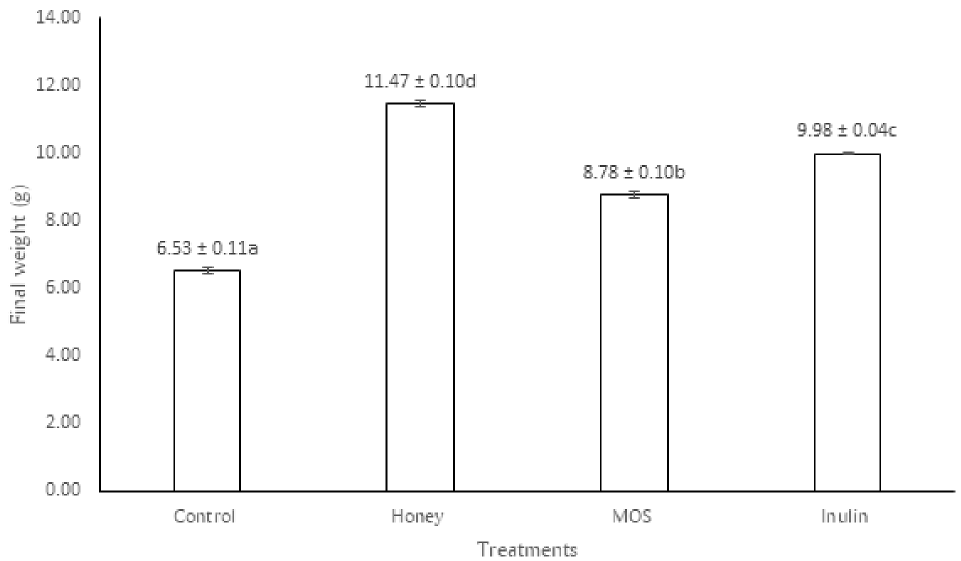

Figure 1. The final weight of Pacific white shrimp administered with different dietary prebiotics. Each data is shown as mean ± standard deviation. Different letters on bars indicate significantly different results (P<0.05)

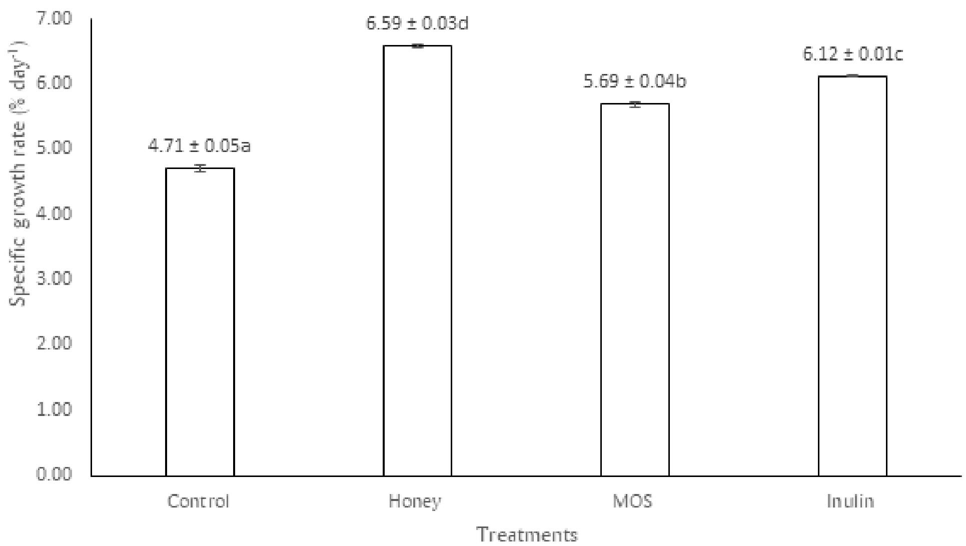

Figure 2. The specific growth rate of Pacific white shrimp administered with different dietary prebiotics. Each data is shown as mean ± standard deviation. Different letters on bars indicate significantly different results (P<0.05)

argued here that MOS has a role in modifying microvilli structure that leads to better nutrient absorption and growth promotion in the Pacific white shrimp (Zhang *et al.*, 2012). Pacific white shrimp fed on feed supplemented with inulin showed higher final weight, weight gain, and specific growth rate than the control shrimp. The improvement in growth performance from the supplementation of inulin is possibly related to nutrient enhancement and availability improved by the increased digestive enzyme (amylase) activity (Zhou *et al.*, 2020). Several natural prebiotics contain several compounds that have a potential as prebiotics. Honey contains oligosaccharides, inulin, FOS, and GOS that have been widely studied for their potential as prebiotics and growth promoters in aquaculture (Abdel-Tawwab, 2016; Davani-Davari *et al.*, 2019). The mixture of prebiotics contained in honey may cause a higher growth in Pacific white shrimp than a single prebiotic. The supplementation of honey to Pacific white shrimp also increases amylase, protease, and lipase activities (Fuandila *et al.*, 2020), which leads to an increase in nutrient absorption and nutrient utilization efficiency.

Dietary prebiotics on Pacific white shrimp also improved feed efficiency expressed by lower FCR values on the prebiotics-supplemented shrimp compared to the control (1.18 ± 005). The lowest FCR (0.93 ± 0.03) was found on the honey treatment ($P<0.05$) (Figure 3).

Dietary of prebiotics (honey, MOS, and inulin) to Pacific white shrimp produced lower FCR than control. The administration of inulin at doses of 0.1, 0.2, and 0.4% did not significantly affect the FCR and condition factor of Pacific white shrimp among treatments (Zhou *et al.*, 2020). The administration of MOS at doses of 0.2, 0.4, and 0.8% to Pacific white shrimp also did not show significantly different results on FCR among treatments (Prastiti *et al.*, 2018). Those results are in contrast with the results of the present study which found significant effects on FCR among treatments when using different prebiotics. These inconsistencies may be due to the differences in production stage, prebiotic property, dose, application duration, and culture condition (Zhou *et al.*, 2020). The honey supplementation to Pacific white shrimp resulted in a lower FCR than the control

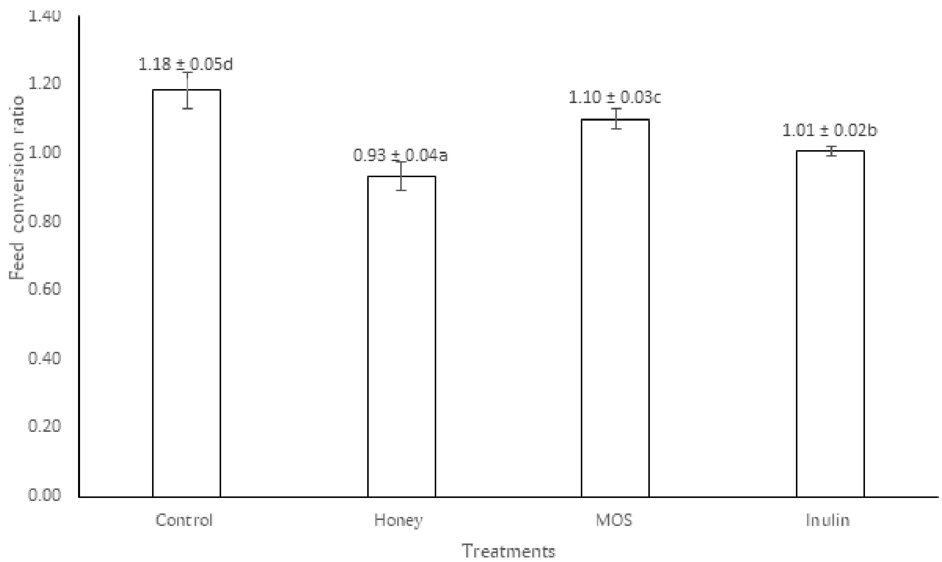

Figure 3. Feed conversion ratio of Pacific white shrimp administered with different dietary prebiotics. Each data is shown as mean ± standard deviation. Different letters on bars indicate significantly different results ($P<0.05$)

(Fuandila *et al.*, 2020). The low FCR indicates an improvement in feed utilization due to increased digestive enzyme activity promoted by the beneficial bacteria in the intestine (Munir *et al.*, 2016). The lowest FCR produced by honey treatment may be caused by the works of the mixture of prebiotics contained in honey. Many types of prebiotic mixtures improve the growth of aquatic animals (Wee *et al.*, 2024). Safari *et al.* (2014) reported that the combination of MOS and FOS provided better growth, feed efficiency, and survival on *Astacus leptodactylus*.

Dietary prebiotics did not significantly affect the survival of Pacific white shrimp (P>0.05). The survival of the experimental shrimp ranged from 86.67% to 93.33% (Figure 4).

The survival of Pacific white shrimp in this study showed no significant differences among treatments. It is in line with the previous studies that reported no significant results from the use of inulin, MOS, and honey on the survival of Pacific white shrimp (Fuandila *et al.*, 2020; Prastiti *et al.*, 2018). These results indicate that the prebiotics used in this study do not cause any deleterious effects on Pacific white shrimp under normal culture conditions.

Intestinal Bacterial Population

The supplementation of honey to the diet of Pacific white shrimp showed a higher total bacterial count in the intestine of Pacific white shrimp (log 6.25 ± 0,17 CFU g^{-1}) than those of other prebiotic treatments (P<0.05). It was not significantly different (P>0.05) from that of control (log 6.39 ± 0.17 CFU g^{-1}) (Figure 5).

The prebiotic used in this study that showed significant effects in the intestinal bacterial population was honey. Pacific white shrimp fed the honey-supplemented diet had a higher total bacterial count than other prebiotics. The supplementation of honey could boost the growth of bacteria in the intestines of Pacific white shrimp (Fuandila *et al.*, 2020; Widanarni *et al.*, 2019b). The increased number of beneficial bacteria in the intestine will enhance digestive enzyme activity, which will lead to the successful application of prebiotics in aquaculture (Hoseinifar *et al.*, 2015).

The supplementation of honey to the diet of Pacific white shrimp also could control the growth of *Vibrio* in the intestine of Pacific

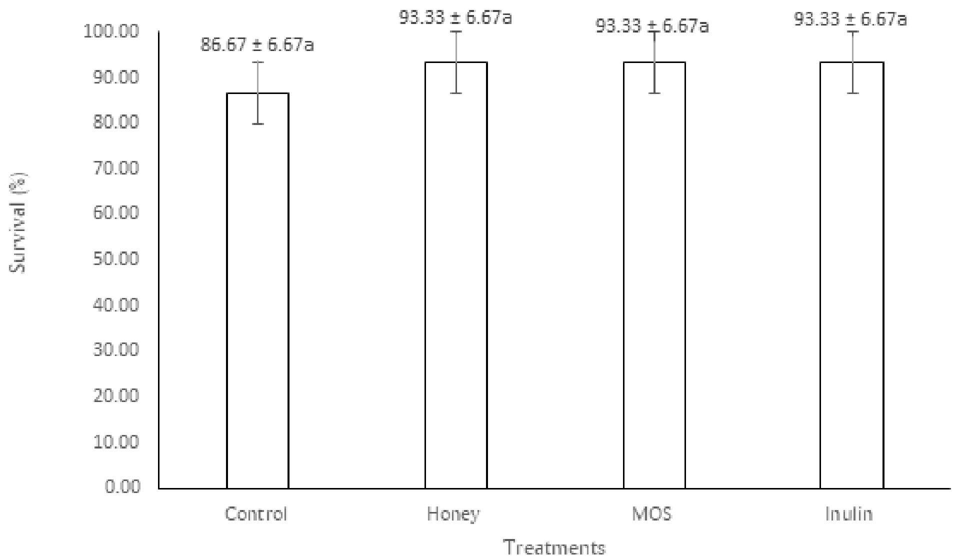

Figure 4. Survival of Pacific white shrimp administered with different dietary prebiotics. Each data is shown as mean ± standard deviation. Different letters on bars indicate significantly different results (P<0.05)

white shrimp. The shrimp fed with the honey-supplemented diet had the lowest total *Vibrio* count (log 3.37 ± 0.26 CFU g^{-1}), which was significantly different ($P<0.05$) from that of the control (log 4.15 ± 0.05 CFU g^{-1}) (Figure 6). The potential of honey to suppress *Vibrio* was also shown by the lowest value in the dominance of *Vibrio* in the Pacific white shrimp intestine (53.85 ± 2.86%) than those of other treatments ($P<0.05$) (Figure 7).

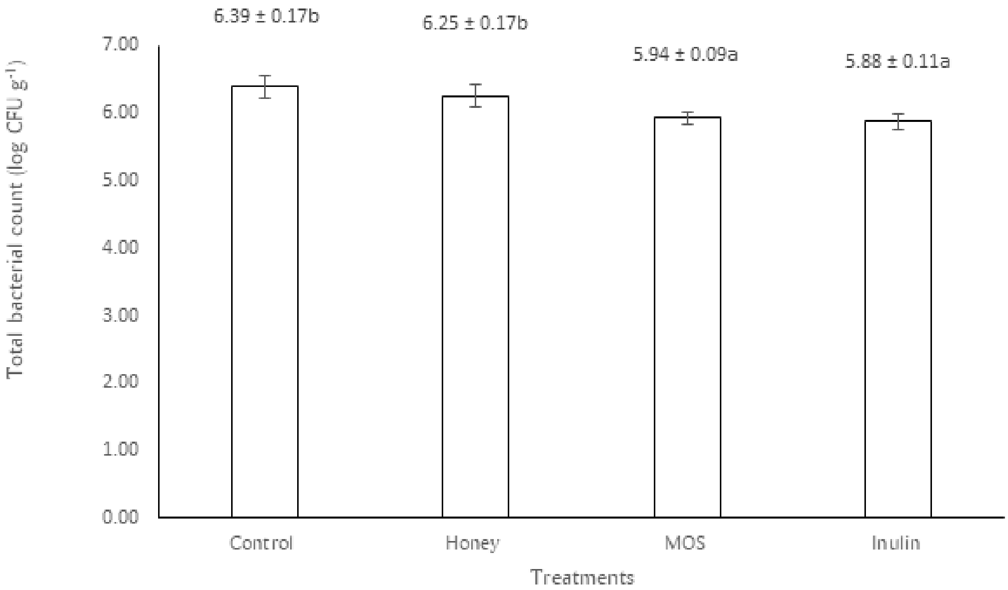

Figure 5. Total bacterial count in the intestine of Pacific white shrimp administered with different dietary prebiotics. Each data is shown as mean ± standard deviation. Different letters on bars indicate significantly different results ($P<0.05$)

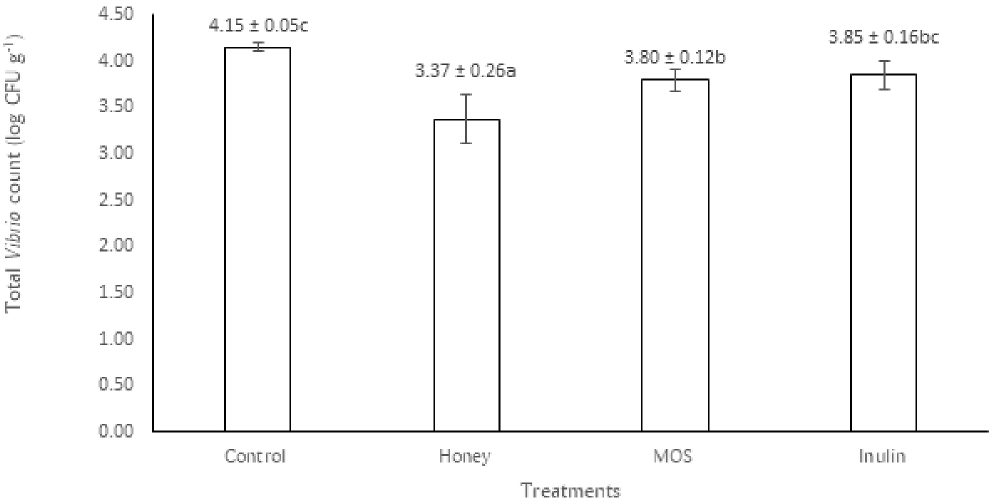

Figure 6. Total *Vibrio* count in the intestine of Pacific white shrimp administered with different dietary prebiotics. Each data is shown as mean ± standard deviation. Different letters on bars indicate significantly different results ($P<0.05$)

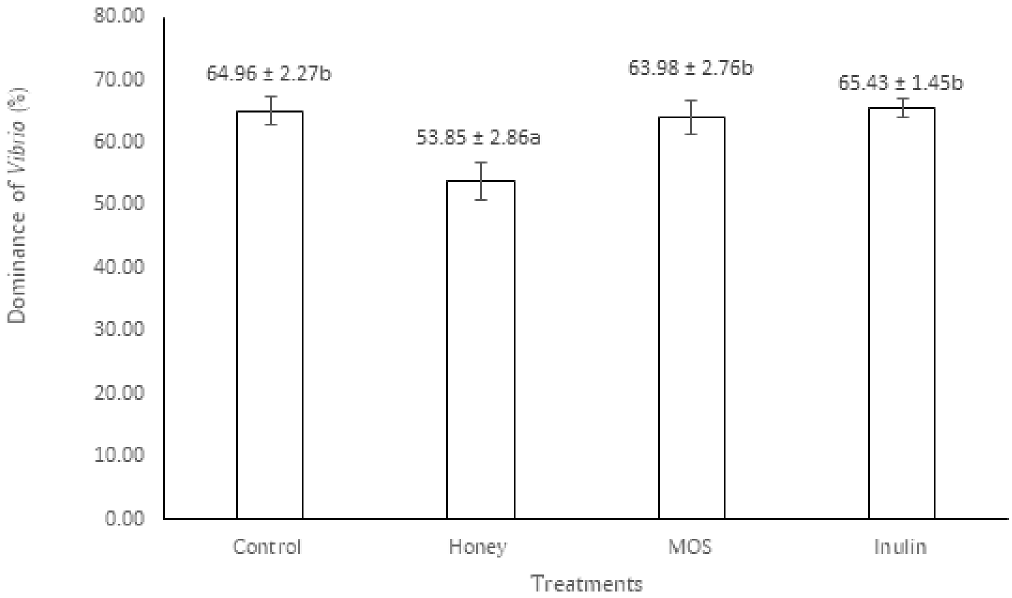

Figure 7. Dominance of *Vibrio* in the intestine of Pacific white shrimp administered with different dietary prebiotics. Each data is shown as mean ± standard deviation. Different letters on bars indicate significantly different results (P<0.05)

The potential of honey to suppress pathogenic bacteria was proven by the lowest total *Vibrio* count and the lowest dominance of *Vibrio* in the intestine of Pacific white shrimp among treatments. Oligosaccharides are fermented by beneficial bacteria and not used effectively by pathogenic bacteria (Yousefian & Amiri, 2009). This fermentation process produces short-chain fatty acids (SCFAs) consisting of lactic acid, propionic acid, and butyric acid (Davani-Davari *et al.*, 2019). These SCFAs are utilized by beneficial bacteria that will make beneficial bacteria more dominant in the gastrointestinal tract of the host (Li *et al.*, 2015). The addition of honey can increase the population of lactic acid bacteria, which will decrease the growth of pathogenic bacteria (Mohan *et al.*, 2017). Moreover, honey also plays a role as an antimicrobial agent due to the production of hydrogen peroxide that inhibits the growth of pathogenic bacteria (Molan, 1992; Schell *et al.*, 2022). The growth stimulation of beneficial bacteria due to the administration of honey will produce bacteriocin that will reduce the population of pathogenic bacteria (Widanarni *et al.*, 2019b).

CONCLUSION

Dietary prebiotics improve the growth performances of Pacific white shrimp. The highest growth performances were found in Pacific white shrimp treated with dietary honey. The improvement in growth performance may be due to the ability of honey to boost the proliferation of beneficial bacteria and suppress the growth of pathogenic bacteria in the intestines of Pacific white shrimp.

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REFERENCES

Abdel-Latif, H. M. R., Yilmaz, E., Dawood, M. A. O., Ringø, E., Ahmadifar, E., & Yilmaz, S. (2022). Shrimp vibriosis and possible control measures using probiotics, postbiotics, prebiotics, and synbiotics: A review. *Aquaculture*, *551*, 737951. https://doi.org/10.1016/j.aquaculture.2022.737951

Abdel-Tawwab, M. (2016). *Feed supplementation to freshwater fish: Experimental approaches*. LAP LAMBERT Academic Publishing.

Al-Sheraji, S. H., Ismail, A., Manap, M. Y., Mustafa, S., Yusof, R. M., & Hassan, F. A. (2013). Prebiotics as functional foods: A review. *Journal of Functional Foods*, *5*(4), 1542-1553. https://doi.org/10.1016/j.jff.2013.08.009

Badan Standardisasi Nasional. (2015). *SNI 2332.3:2015 cara uji mikrobiologi – bagian 3: Penentuan angka lempeng total (ALT) pada produk perikanan*. Badan Standardisasi Nasional.

Bossier, P., De Schrijver, P., Defoirdt, T., Ruwandeepika, H. A. D., Natrah, F., Ekasari, J., Toi, H., Nhan, D., Tinh, N., Pande, G., Karunasagar, I., & Van Stappen, G. (2016). Microbial community management in aquaculture. *Procedia Food Science*, *6*, 37-39. https://doi.org/10.1016/j.profoo.2016.02.007

Cornejo-Granados, F., Gallardo-Becerra, L., Leonardo-Reza, M., Ochoa-Romo, J. P., & Ochoa-Leyva, A. (2018). A meta-analysis reveals the environmental and host factors shaping the structure and function of the shrimp microbiota. *PeerJ*, *6*, e5382. https://doi.org/10.7717/peerj.5382

Cornejo-Granados, F., Lopez-Zavala, A. A., Gallardo-Becerra, L., Mendoza-Vargas, A., Sánchez, F., Vichido, R., Brieba, L. G., Viana, M. T., Sotelo-Mundo, R. R., & Ochoa-Leyva, A. (2017). Microbiome of Pacific whiteleg shrimp reveals differential bacterial community composition between wild, aquacultured and AHPND/EMS outbreak conditions. *Scientific Reports*, *7*, 11783. https://doi.org/10.1038/s41598-017-11805-w

Davani-Davari, D., Negahdaripour, M., Karimzadeh, I., Seifan, M., Mohkam, M., Masoumi, S. J., Barenjian, A., & Ghasemi, Y. (2019). Prebiotics: Definition, types, sources, mechanisms, and clinical applications. *Foods*, *8*(3), 92. https://doi.org/10.3390/foods8030092

Duan, Y., Wang, Y., Liu, Q., Dong, H., Li, H., Xiong, D., & Zhang, J. (2019). Changes in the intestine microbial, digestion and immunity of *Litopenaeus vannamei* in response to dietary resistant starch. *Scientific Reports*, *9*, 6464. https://doi.org/10.1038/s41598-019-42939-8

Ebrahimi, G., Ouraji, H., Khalesi, M.K., Sudagar, M., Barari, A., Dangesaraki, M. Z. & Khalili, K. H. J. (2012). Effects of a prebiotic, Immunogen®, on feed utilization, body composition, immunity and resistance to *Aeromonas hydrophila* infection in the common carp *Cyprinus carpio* (Linnaeus) fingerlings. *Journal of Animal Physiology and Animal Nutrition*, *96*(4), 591-599. https://doi.org/10.1111/j.1439-0396.2011.01182.x

Fuandila, N. N., Widanarni, & Yuhana, M. (2020). Growth performance and immune response of prebiotic honey fed Pacific white shrimp *Litopenaeus vannamei* to *Vibrio parahaemolyticus* infection. *Journal of Applied Aquaculture*, *32*(3), 221-235. https://doi.org/10.1080/10454438.2019.1615593

Gibson, G. R., Hutkins, R., Sanders, M. E., Prescott, S. L., Reimer, R. A., Salminen, S. J., Scott, K., Stanton, C., Swanson, K. S., Cani, P. D., Verbeke, K., & Reid, G. (2017). Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nature Reviews Gastroenterology & Hepatology*, *14*, 491-502. https://doi.org/10.1038/nrgastro.2017.75

Hoseinifar, S. H., Khalili, M., Rufchaei, R., Raeisi, M., Attar, M., Cordero, H., & Esteban, M. Á. (2015). Effects of date palm fruit extracts on skin mucosal immunity, immune related genes expression and growth performance of common carp (*Cyprinus carpio*) fry. *Fish & Shellfish Immunology*, *47*(2), 706-711. https://doi.org/10.1016/j.fsi.2015.09.046

Hu, X., Yang, H. L., Yan, Y. Y., Zhang, C. X., Ye, J. D., Lu, K. L., Hu, L. H., Zhang, J. J., Ruan, L., & Sun, Y. Z. (2019). Effects of fructooligosaccharide on growth, immunity and intestinal microbiota of shrimp (*Litopenaeus vannamei*) fed diets with fish meal partially replaced by soybean meal. *Aquaculture Nutrition*, *25*(1), 194-204. https://doi.org/10.1111/anu.12843

Jory, D. (2023). *Annual farmed shrimp production survey: A slight decrease in production reduction in 2023 with hopes for renewed growth in 2024. Responsible Seafood Advocate*. Global Seafood Alliance.

Koh, A., De Vadder, F., Kovatcheva-Datchary, P., & Bäckhed, F. (2016). From dietary fiber to host physiology: short-chain fatty acids as key bacterial metabolites. *Cell*, *165*(6), 1332-1345. https://doi.org/10.1016/j.cell.2016.05.041

Levy, M., Blacher, E., & Elinav, E. (2017). Microbiome, metabolites and host immunity. *Current Opinion in Microbiology*, *35*, 8-15. https://doi.org/10.1016/j.mib.2016.10.003

Li, W., Wang, K., Sun, Y., Ye, H., Hu, B., & Zeng, X. (2015). Influences of structures of galactooligosaccharides and fructooligosaccharides on the fermentation in vitro by human intestinal microbiota. *Journal of Functional Food*, *13*, 158-168. https://doi.org/10.1016/j.jff.2014.12.044

Li, Y., Liu, H., Dai, X., Li, J., & Ding, F. (2018). Effects of dietary inulin and mannan oligosaccharide on immune related genes expression and disease resistance of Pacific white shrimp, *Litopenaeus vannamei*. *Fish & Shellfish Immunology*, *76*, 78-92. https://doi.org/10.1016/j.fsi.2018.02.034

Li, Y., Yuan, W., Zhang, Y., Liu, H., & Dai, X. (2021). Single or combined effects of dietary arabinoxylan-oligosaccharide and inulin on growth performance, gut microbiota, and immune response in Pacific white shrimp *Litopenaeus vannamei*. *Journal of Oceanology and Limnology*, *39*, 741-754. https://doi.org/10.1007/s00343-020-9083-z

Li, Z., Tran, N. T., Ji, P., Sun, Z., Wen, X., & Li, S. (2019). Effects of prebiotic mixtures on growth performance, intestinal microbiota and immune response in juvenile chu's croaker, *Nibea coibor*. *Fish & Shellfish Immunology*, *89*, 564-573. https://doi.org/10.1016/j.fsi.2019.04.025

Mohammadi, G., Hafezieh, M., Karimi, A. K., Azra, M. N., Doan, H. V., Tapingkae, W., Abdelrahman, H. A., & Dawood, M. A. O. (2022). The synergistic effects of plant polysaccharide and *Pediococcus acidilactici* as a symbiotic additive on growth, antioxidant status, immune response, and resistance of Nile tilapia (*Oreohcromis niloticus*) against *Aeromonas hydrophila*. *Fish & Shellfish Immunology*, *120*, 304-313. https://doi.org/10.1016/j.fsi.2021.11.028

Mohan, A., Quek, S.-Y., Gutierrez-Maddox, N., Gao, Y., & Shu, Q. (2017). Effect of honey in improving the gut microbial balance. *Food Quality and Safety*, *1*(2), 107-115. https://doi.org/10.1093/fqsafe/fyx015

Molan, P. C. (1992). The antibacterial activity of honey: 1. The nature of the antibacterial activity. *Bee World*, *73*(1), 5-28. https://doi.org/10.1080/0005772X.1992.11099109

Munir, M. B., Hashim, R., Manaf, M. S. A., & Nor, S. A. M. (2016). Dietary prebiotics and probiotics influence the growth performance, feed utilization, and body indices of snakehead (*Channa striata*) fingerlings. *Tropical Life Sciences Research*, *27*(2), 111-125. https://doi.org/10.21315/tlsr2016.27.2.9

Ochoa-Romo, J. P., Cornejo-Granados, F., Lopez-Zavala, A. A., Viana, M. T., Sánchez, F., Gallardo-Becerra, L., Luque-Villegas, M., Valdez-López, Y., Sotelo-Mundo, R. R., Cota-Huízar, A., López_Munguia, A., & Ochoa-Leyva, A. (2022). Agavin induces beneficial microbes in the shrimp microbiota under farming conditions. *Scientific Reports*, *12*, 6392. https://doi.org/10.1038/s41598-022-10442-2

Prastiti, L. A., Yuhana, M., & Widanarni. (2018). Effectivity of prebiotic mannan oligosaccharides as the immunity enhancer and growth response on whiteleg shrimp *Litopenaeus vannamei* against white spot disease. *Jurnal Akuakultur Indonesia*, *17*(1), 81-86. https://doi.org/10.19027/jai.17.1.81-86

Safari, O., Shahsavani, D., Paolucci, M., & Atash, M. M. S. (2014). Single or combined effects of fructo- and mannan oligosaccharide supplements on the growth performance, nutrient digestibility, immune response and stress resistance of juvenile narrow clawed crayfish, *Astacus leptodactylus* Eschscholtz, 1823. *Aquaculture*, *432*, 192-203. https://doi.org/10.1016/j.aquaculture.2014.05.012

Sang, H. M., Kien, N. T., & Thanh Thuy, N. T. (2014). Effect of dietary mannan oligosaccharide on growth, survival, physiological, immunological and gut morphological conditions of black tiger prawn (*Penaeus monodon* Fabricius 1798). *Aquaculture Nutrition*, *20*(3), 341-348. https://doi.org/10.1111/anu.12083

Schell., K. R., Fernandes, K. E., Shanahan, E., Wilson, I., Blair, S. E., Carter, D. A., & Cokcetin, N. N. (2022). The potential of honey as a prebiotic food to re-engineer the gut microbiome toward a healthy state. *Frontiers in Nutrition*, *9*, 957932. https://doi.org/10.3389/fnut.2022.957932

Shoaei, R., Akrami, R., Ghobadi, S., & Mansour, M. R. (2015). Effect of dietary of prebiotic mannan oligosaccharide and β-1,3 glucan on growth performance, survival, body composition and serum lysozyme activity in rainbow trout (*Oncorhynchus mykiss*) fingerling. *Journal of Marine Biology*, *7*(2), 45-56.

Song, S. K., Beck, B. R., Kim, D., Park, J., Kim, J., Kim, H. D., & Ringø, E. (2014). Prebiotics as immunostimulants in aquaculture: A review. *Fish & Shellfish Immunology*, *40*(1), 40-48. https://doi.org/10.1016/j.fsi.2014.06.016

Stentiford, G. D., Sritunyalucksana, K., Flegel, T. W., Williams, B. A. P., Withyachumnarnkul, B., Itsathitphaisarn, O., & Bass, D. (2017). New paradigms to help solve the global aquaculture disease crisis. *Plos Pathogens*, *13*(2), e1006160. https://doi.org/10.1371/journal.ppat.1006160

Vadstein, O., Bergh, Ø., Gatesoupe, F. J., Galindo-Villegas, J., Mulero, V., Picchietti, S., Scapigliati, G., Makridis, P., Olsen, Y., Dierckens, K., Defoirdt, T., Boon, N., De Schryver, P., & Bossier, P. (2013). Microbiology and immunology of fish larvae. *Reviews in Aquaculture*, *5*(1), S1-S25. https://doi.org/10.1111/j.1753-5131.2012.01082.x

Wee, W., Hamid, N. K. A., Mat, K., Khalif, R. I. A. R., Rusli, N. D., Rahman, M. M., Kabir, M. A., & Wei, L. S. (2024). The effects of mixed prebiotics in aquaculture: A review. *Aquaculture and Fisheries*, *9*(1), 28-34. https://doi.org/10.1016/j.aaf.2022.02.005

Widanarni, Gustilatov, M., Sukenda, & Utami, D. A. S. (2019a). Utilization of honey to improve immune response and resistance of Pacific white shrimp (*Litopenaeus vannamei*) against white spot syndrome virus infection. *Jurnal Riset Akuakultur*, *14*(1), 59-69. http://dx.doi.org/10.15578/jra.14.1.2019.59-69

Widanarni, Putri, F. N., & Rahman. (2019b). *Growth performance of white shrimp Litopenaeus vananmei fed with various dosages of prebiotic honey. IOP Conference Series: Earth and Environmental Science, 278*, 012079. https://doi.org/10.1088/1755-1315/278/1/012079

Widanarni, Rahmi, D., Gustilatov, M., Sukenda, & Utami, D. A. S. (2020). Immune responses and resistance of white shrimp *Litopenaeus vannamei* administered *Bacillus* sp. NP5 and honey against white spot syndrome virus infection. *Jurnal Akuakultur Indonesia*, *19*(2), 118-130. https://doi.org/10.19027/jai.19.2.118-130

Yilmaz, S., Yilmaz, E., Dawood, M. A. O., Ringø, E., Ahmadifar, E., & Abdel-Latif, H. M. R. (2022). Probiotics, prebiotics, and synbiotics used to control vibriosis in fish: A review. *Aquaculture*, *547*, 737514. https://doi.org/10.1016/j.aquaculture.2021.737514

Yousefian, M., & Amiri, M. S. (2009). A review of the use of prebiotic in aquaculture for fish and shrimp. *African Journal of Biotechnology*, *8*(25), 7313-7318.

Zhou, L., Li, H., Qin, J.G., Wang, X., Chen, L., Xu, C., & Li, E. (2020). Dietary prebiotic inulin benefits on growth performance, antioxidant capacity, immune response and intestinal microbiota in Pacific white shrimp (*Litopenaeus vannamei*) at low salinity. *Aquaculture*, *518*, 734847. https://doi.org/10.1016/j.aquaculture.2019.734847

