The growth, survival and intestinal bacteria in white shrimp (*Litopenaeus vannamei*) with addition of probiotics, prebiotics and synbiotics in Feeds

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Abstract

White shrimp (*Litopenaeus vannamei*) aquaculture faces many disease problems. The use of antibiotics to treat diseases leads to the development of antibiotic-resistant bacteria, which can endanger human health. To overcome this problem, probiotics, prebiotics and synbiotics are eco-friendly solutions that can be used in shrimp aquaculture to improve water quality, growth, survival and disease resistance.

This study aimed to determine the effect of probiotics, prebiotics, and synbiotics on growth, survival, and intestinal bacteria in white shrimp (*L. vannamei*). The experiment was conducted using a laboratory experimental method with a completely randomized design consisted of 4 treatments and 4 replications. (K) Without supplementation of probiotics, prebiotics, and synbiotics in feed; (Pro) Supplementation of probiotics in feed; (Pre) Supplementation of prebiotics in feed; and (Syn) Supplementation of synbiotics in feed. The parameters measured were SGR, FCR, SR, total lactic acid bacteria and vibrio dominance.

The data were analyzed using One-Way ANOVA with SPSS version 25 and a 95% confidence interval. If the results had a significant effect (P<0,05), Duncan's test was used to determine differences between treatments. The results showed that probiotic and synbiotic treatments gave the best results on SGR, FCR, SR, total lactic acid bacteria and vibrio dominance compared to the control treatment.

This study concluded that added probiotics and synbiotics in feed can increase total lactic acid bacteria and reduce the dominance of vibrio in the intestines of white shrimp, as well as increase the growth and survival of white shrimp (*L. vannamei*).

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Keywords: *Litopenaeus vannamei*, *prebiotics*, *probiotics*, *synbiotics*

Introduction

Aquaculture is an important fisheries sector as it contributes to national food security, people's income, employment development, and foreign exchange earnings (Amelia *et al.*, 2021) [1]. White shrimp aquaculture (*Litopenaeus vannamei*) is one of the main fisheries sector in Indonesia. However, the development of white shrimp aquaculture has many disease problems, resulting in decreased production and significant economic losses.

The use of antibiotics to address disease problems in shrimp aquaculture has become a major concern due to the development of Antimicrobial Resistance (AMR) in bacteria. The widespread dissemination of antibiotic resistant bacteria from shrimp ponds poses potential risks to human health and the environment (Nadella *et al.*, 2021) [10]. Antimicrobial Resistance (AMR) is the ability of microbial organisms to resist the effects of drugs and chemicals designed to kill them (Thornber *et al.*, 2019) [15]. The findings of Nadella *et al*. (2021) [10] stated that the highest resistance to oxytetracycline antibiotics was observed in bacteria isolated from shrimp samples compared to bacteria from sediment and water samples. The spread of antibiotic resistant bacteria in shrimp carries significant health risks for humans consuming them.

Probiotics and prebiotics are increasingly being used as eco-friendly solutions in shrimp aquaculture to improve water quality, growth, survival rates, and disease resistance (Noman *et al*., 2014). Probiotics are live microorganisms that can be administered through feed to cultured organisms to provide benefits such as improved digestion, growth, and immune response. Prebiotics are non-digestible foods that stimulate the activity of beneficial bacteria in the intestines, thereby providing benefits to the host (Hasyimi *et al*., 2020) [7], while synbiotics are a combination of probiotics and prebiotics (Huynh *et al*., 2017) [8]

Probiotics are commonly used in aquaculture to protect cultured organisms from pathogens and improve water quality and feed efficiency (El-Saadony *et al*., 2022) [5]. However, there is limited information available on the benefits of using prebiotics, such as inulin, in shrimp aquaculture (Zhou *et al*., 2020) [22], as well as synbiotics (Okey *et al*., 2018; Noman *et al*., 2024) [11]. Therefore, further study is needed to determine the effect of probiotics, prebiotics and synbiotics on aquaculture, especially white shrimp (*L. vannamei*) aquaculture.

Materials and Method

Experimental design

The method used in this study is laboratory experimental method. The experimental design used was a completely randomized design (CRD) with 4 treatments and 4 replications. The treatments were: (K) Without supplementation of probiotics, prebiotics, and synbiotics in feed; (Pro) Supplementation of probiotics in feed; (Pre) Supplementation of prebiotics in feed; and (Syn) Supplementation of synbiotics in feed.

Experimental media

The plastic rearing containers, measuring 55x35x30 cm, were cleaned, washed, and sterilized with a 40 ppm dose of chlorine. After setting up aeration equipment, 35 L of seawater, which had passed the filtration process, was added to each container.

Experimental shrimp

The white shrimp (*L. vannamei*) used in this study were obtained from Jepara Brackish Water Aquaculture Center (BBPBAP) with an average weight 3-4 g/shrimp.

Experimental diets

The experimental feed consisted of commercial pellets containing 35% protein. The probiotic bacteria used were *lactobacillus*, *bifidobacterium*, and *micrococcus* from shrimp intestines. The probiotic dose used was 1% of the total feed with a bacterial density of 10^8 cfu/mL (Widanarni *et al*., 2020). The prebiotic used was inulin at a dose of 0,4% of the total feed (Zhou *et al*., 2020) [22], while the synbiotic was a combination of 1% probiotics and 0,4% prebiotics. Feed mixing is done by adding supplements according to the treatment with egg yolk 2% of the total feed as an adhesive (Widanarni *et al*., 2020) [7].

Shrimp rearing

White shrimp with an average weight of 3-4 g/shrimp were stocked in experimental containers at a stocking density of 15 shrimp per container. White shrimp were reared for 30 days and fed with experimental diets according to each treatment, four times a day (Zainuddin *et al*., 2019) [21], with a dose of 15% of shrimp weight according to the Indonesian National Standard (SNI) 01-7246-2006.

Water quality is maintained in optimal conditions by performing water changes every morning by 10-20%. Water quality parameters such as dissolved oxygen (DO), temperature, pH and salinity are measured once a week and maintained under conditions of dissolved oxygen >3.5 ppm, temperature ranging from 28 to 31°C, pH ranging from 7,5 to 8,0, and salinity ranging from 30 to 34 ppt.

Experimental parameters

1. SGR (*Spesific Growth Rate*)

The Specific Growth Rate (SGR) was calculated using the following formula (Xue *et al*., 2021) [19]:

$$SGR = \frac{\ln Wt - \ln Wo}{t} \times 100\ \%$$

SGR = Spesific Growth Rate (%/day)
Wt = Final average body weight (g)
Wo = Initial average body weight (g)
t = Experimental time (day)

2. FCR (*Food Conversion Ratio*)

The Food Conversion Ratio (FCR) was calculated using the following formula (Widanarni *et al*., 2014):

$$FCR = \frac{F}{Bt + Bm - Bo}$$

FCR = Food Conversion Ratio
F = Total feed given (g)
Bt = Final biomass of shrimp (g)
Bm = Died biomass during rearing (g)
Bo = Initial biomass of shrimp (g)

3. SR (*Survival Rate*)

The Survival Rate (SR) data was calculated using the following formula (Zainuddin *et al*., 2019) [21]:

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

SR = Survival Rate (%)
Nt = Final number of shrimps
No = Initial number of shrimps

4. Total lactic acid bacteria

Total lactic acid bacteria were counted by the total plate count (TPC) method. Samples of shrimp intestine in each treatment were taken and homogenized, then serially diluted. Samples up to 0,1 ml were cultured in MRS (de Man, Rogosa, and Sharpe) media. The samples were incubated for 24 to 48 hours and the growing colonies were counted.

5. Vibrio dominance

Vibrio dominance was calculated by comparing total vibrio and total bacteria in the shrimp intestines. Bacterial counts were performed using the total plate count (TPC) method. Samples of shrimp intestine in each treatment were taken and homogenized, then serially diluted. Samples up to 0,1 mL were cultured in NA (Nutrient Agar) media for total bacteria and TCBS (Thiosulfate Citrate Bile Salts Sucrose) media for total Vibrio. The samples were incubated for 24 hours and the growing colonies were counted.

The vibrio dominance was calculated using the following formula:

$$Dominasi\ vibrio\ (\%) = \frac{Total\ vibrio}{Total\ bakteri}\ x\ 100\ \%$$

Data Analysis

Observed data were specific growth rate (SGR), feed conversion ratio (FCR), survival rate (SR), total lactic acid bacteria, and Vibrio dominance. Data were analyzed by one-way analysis of variance (ANOVA) with SPSS software version 25 and 95% confidence interval to determine the effect between treatments. When there was a significant difference (P<0,05), Duncan's test was used to determine the difference between treatments.

Results

Spesific Growth Rate (SGR)

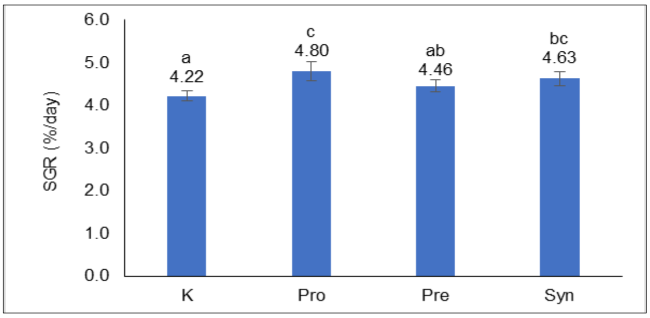

Fig 1: SGR of white shrimp

The SGR at the end of rearing ranged from 4,22 to 4,80%, presented in Figure 1. The highest SGR was obtained in the probiotic treatment and the lowest SGR was obtained in the control treatment. ANOVA results showed that there were significant differences between treatments (P<0,05), and further Duncan's test showed that probiotics (4,80 ± 0,22%) and synbiotics (4,63 ± 0,16%) treatments were significantly different compared to the control (4,22 ± 0,12%), but prebiotic treatment (4,46 ± 0,14%) was not significantly different compared to the control. This showed that the supplementation of probiotics and synbiotics had a significant effect on increasing the SGR of white shrimp, but the supplementation of prebiotics had no significant effect on increasing the SGR of white shrimp.

Food Conversion Ratio (FCR)

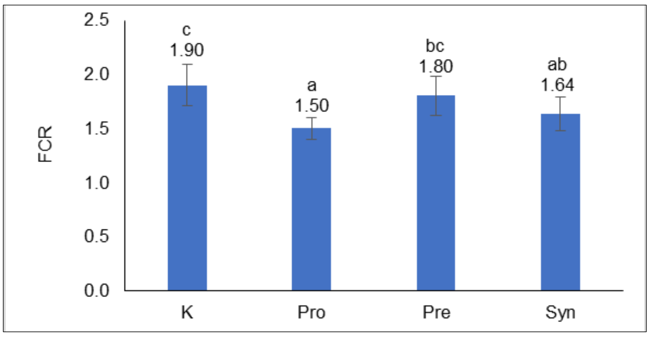

Fig 2: FCR of white shrimp

FCR at the end of rearing ranged from 1,50 to 1,90, presented in Figure 2. The lowest FCR was obtained in the probiotic treatment and the highest FCR was obtained in the control treatment. ANOVA results showed that there were significant differences between treatments (P<0,05), and further Duncan's test showed that probiotic (1,50 ± 0,10) and synbiotic (1,64 ± 0,16) treatments were significantly different compared to the control (1,90 ± 0,19), but prebiotic treatment (1,80 ± 0,18) was not significantly different compared to the control. This showed that the supplementation of probiotics and synbiotics had a significant effect on reducing FCR of white shrimp, but the supplementation of prebiotics had no significant effect on reducing FCR of white shrimp.

***Survival Rate* (SR)**

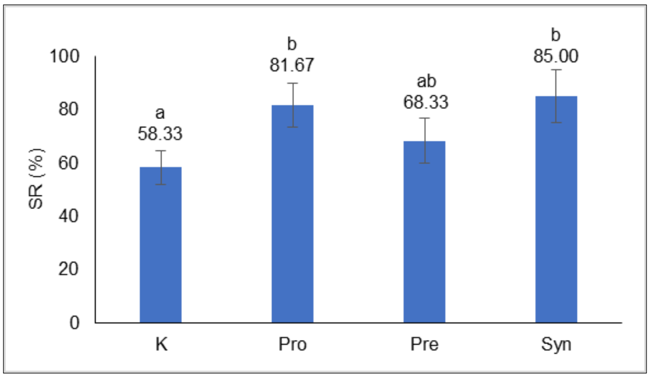

Fig 3: SR of white shrimp

The survival rate (SR) of white shrimp at the end of rearing ranged from 58,33 to 85,00%, presented in Figure 3. The highest SR was obtained in the synbiotic treatment and the lowest SR was obtained in the control treatment. ANOVA results showed that there were significant differences between treatments (P<0,05), and further Duncan's test showed that SR in the treatment of synbiotics (85,00 ± 10,00%) and probiotics (81,67 ± 8,39%) was significantly different compared to the control (58,33 ± 6,38%), but prebiotic treatment (68,33 ± 8,39%) was not significantly different compared to the control. This showed that the supplementation of synbiotics and probiotics in the diet had a significant effect on increasing the SR of white shrimp, while the supplementation of prebiotics had no significant effect on increasing the SR of white shrimp.

Total Lactic Acid Bacteria

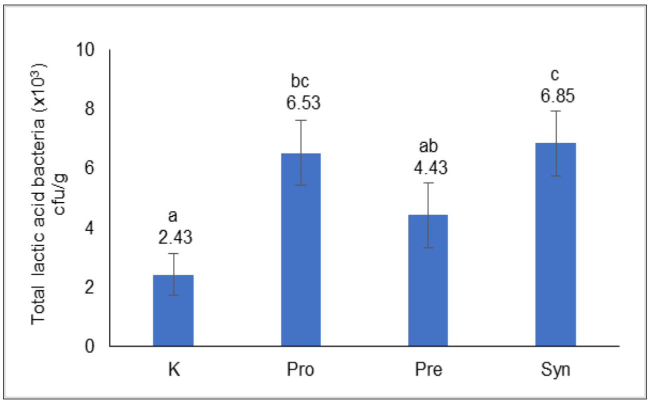

Fig 4: Total lactic acid bacteria in the intestines of white shrimp

Total lactic acid bacteria in the intestines of white shrimp at the end of rearing ranged from 2,43x10^3 to 6,85x10^3 cfu/g, presented in Figure 4. The highest total lactic acid bacteria was obtained in the synbiotic treatment and the lowest total lactic acid bacteria was obtained in the control treatment. ANOVA results showed that there were significant differences between treatments (P<0,05), and further Duncan's test showed that the synbiotic (6,85x10^3 ± 1,10x10^3 cfu/g) and probiotics (6,53x10^3 ± 1,09x10^3 cfu/g) treatments were significantly different compared to the control (2.43x10^3 ± 0.71x10^3 cfu/g), but the prebiotic treatment (4,43x10^3 ± 1,11x10^3 cfu/g) was not significantly different compared to the control. This showed that the supplementation of probiotics and synbiotics in feed had a significant effect on increasing the number of lactic acid bacteria in the intestines of white shrimp, but the supplementation of prebiotics had no significant effect on increasing the number of lactic acid bacteria in the intestines of white shrimp.

Vibrio dominance

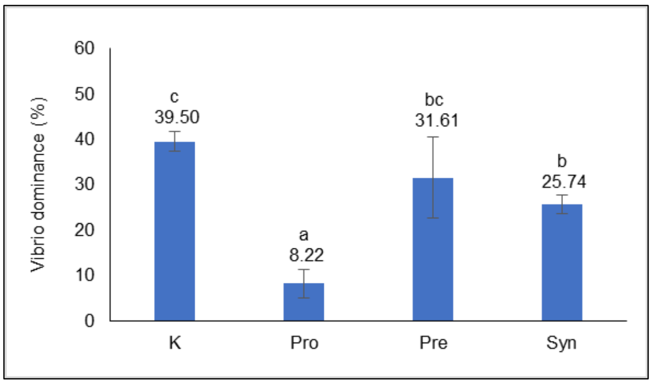

Fig 5: Vibrio dominance in the intestines of white shrimp

Vibrio dominance in the intestines of white shrimp at the end of rearing ranged from 8,22 to 39,50%, presented in figure 5. The lowest vibrio dominance was obtained in the probiotic treatment and the highest vibrio dominance was obtained in the control treatment. ANOVA results showed that there were significant differences between treatments (P<0,05), and further Duncan's test showed that probiotic (8,22 ± 3,04%) and synbiotic (25,74 ± 2,02%) treatments were significantly different compared to the control (39,50 ± 2,19%), but prebiotic treatment (31,61 ± 8,97%) was not significantly different compared to the control. There was a significant difference between probiotic and synbiotic treatments. This showed that the supplementation of probiotics and synbiotics had a significant effect on reducing the dominance of vibrio in the intestines of white shrimp, but the supplementation of prebiotics had no significant effect on reducing the dominance of vibrio in the intestines of white shrimp. The best vibrio dominance was obtained in the probiotic treatment.

Discussion

The growth parameters observed in this study were SGR and FCR. The highest SGR (4,80%) was obtained in the probiotic treatment and the lowest SGR (4,22%) was obtained in the control treatment. Probiotic (4,80 ± 0,22%) and synbiotic (4,63 ± 0,16%) treatments significantly increased the SGR of white shrimp compared to the control (4,22 ± 0,12%), but prebiotic treatment (4,46 ± 0,14%) had no significant effect on SGR compared to the control.

Other growth parameters are FCR. The lowest FCR (1,50) was obtained in the probiotic treatment and the highest FCR (1,90) was obtained in the control treatment. Probiotic (1,50 ± 0,10) and synbiotic (1,64 ± 0,16) treatments significantly reducing the FCR of white shrimp compared to the control (1,90 ± 0,19), but prebiotic treatment (1,80 ± 0,18) had no significant effect on FCR compared to the control.

The results of this study on the survival of white shrimp were the highest SR (85,00%) obtained in the synbiotic treatment and the lowest SR (58,33%) obtained in the control treatment. Similar results to growth were also obtained for white shrimp survival that synbiotic (85,00 ± 10,00%) and probiotic (81,67 ± 8,39%) treatments significantly increased the SR of white shrimp compared to the control (58,33 ± 6.38%), but the prebiotic treatment (68,33 ± 8,39%) had no significant effect on SR compared to the control. This study showed that the supplementation of probiotics and synbiotics in the diet had a significant effect on increasing growth and survival of white shrimp, but the application of prebiotics had no significant effect on growth and survival of white shrimp.

The best growth and survival in the probiotic and synbiotic treatments compared to the prebiotic treatment thought to be due to the content of probiotic bacteria in both treatments. Probiotics are beneficial bacteria that can be added through feed and can increase shrimp immunity to disease and increase shrimp survival. The addition of probiotics in the feed can improve growth performance, such as SGR and FCR, and also the survival of penaeid shrimp (Toledo *et al.*, 2019) [16].

The survival of white shrimp in the prebiotic treatment did not show any significant difference compared to the control. This could be attributed to the fact that the prebiotic inulin given to the shrimp was not directly digestible. Prebiotics are a food source for probiotic bacteria to stimulate their growth in the shrimp digestive tract, but these prebiotics cannot be directly utilized by shrimp (Okey *et al.*, 2018) [12]. This showed that the use of prebiotic alone without probiotic bacteria in feed was not effective because prebiotics cannot be utilized by shrimp to increase their survival, but different results in synbiotic treatments that used a combination of prebiotics and probiotics still provided high shrimp survival results. Zhou *et al.* (2020) [22] also found that the addition of inulin to the diet at the same dose of 0,4% had no significant effect on shrimp survival.

The predominance of probiotics in the shrimp gut is important (Tseng *et al.*, 2023) [17]. Probiotic bacteria can produce antibacterial compounds to fight pathogenic bacteria (El-Saadony *et al.*, 2022) [5], such as bacteriocins that can aid resistance to certain pathogens and reduce shrimp mortality (Cai *et al.*, 2022) [2]. Bacteriocins, produced by lactic acid bacteria such as *Lactobacillus*, can help shrimp fight pathogens and increase survival (Tseng *et al.*, 2023) [17].

Total lactic acid bacteria in the intestine of white shrimp during rearing ranged from 2,43x10^3 to 6,85x10^3 cfu/g with the highest results in the synbiotic treatment and the lowest results in the control treatment. Synbiotic (6,85x10^3 ± 1,10x10^3 cfu/g) and probiotic (6,53x10^3 ± 1,09x10^3 cfu/g) treatments significantly increased the total lactic acid bacteria compared to the control (2,43x10^3 ± 0,71x10^3 cfu/g), but the prebiotic treatment (4,43x10^3 ± 1,11x10^3 cfu/g) had no significant effect on total lactic acid bacteria compared to the control.

Shrimp fed with the addition of synbiotics had higher total lactic acid bacteria compared to the probiotic treatment. The combined addition of probiotics and prebiotics had a more positive effect than their individual additions (Yao *et al.*, 2021) [20]. However, upon further analysis there was no significant difference between the two treatments. This is thought to be due to the addition of inulin prebiotics in the synbiotic treatment, which can be used by probiotic bacteria to stimulate the development of normal microflora in the digestive system of white shrimp.

Probiotic bacteria mixed in the diet in probiotic and synbiotic treatments are thought to be beneficial in maintaining the population of lactic acid bacteria in the intestines of white shrimp. *Lactobacillus* and *bifidobacterium* bacteria used in this study are two groups of lactic acid bacteria that are widely used as probiotics (Das *et al.*, 2017) [4]. Probiotics can produce protease or lipase enzymes that are beneficial for digestion, improve the efficiency of feed absorption, and enhance growth (Tseng *et al.*, 2023) [17]. Effective digestion allows shrimp to efficiently digest and utilize the nutrients in the feed to maximize their immune system, so shrimp have sufficient energy reserves to effectively fight viruses and diseases and increase shrimp survival (Ghosh, 2023). The findings of Cai *et al.* (2022) stated that four latic acid bacteria *Lactococcus lactis* (strains S1 and S2), *Enterococcus faecali* (strains F3 and F7) significantly improved growth, survival, and disease resistance of *L. vannamei*.

Vibrio dominance in the intestines of white shrimp at the end of rearing ranged from 8,22 to 39,50% with the lowest results obtained in the probiotic treatment and the highest results in the control treatment. Probiotic (8,22 ± 3,04%) and synbiotic (25,74 ± 2,02%) treatments significantly reduced the vibrio dominance compared to the control (39,50 ± 2,19%), but prebiotic treatment (31,61 ± 8,97%) had no significant effect compared to the control.

Vibrio is a pathogenic bacterium that causes many diseases in shrimp (Ramirez *et al.*, 2022) [13]. *Vibrio* sp. bacteria are causative agents of disease when shrimp are stressed and weak, and can cause death. The addition of probiotic bacteria in the diet can increase the number of beneficial bacteria and reduce harmful bacteria in the shrimp digestive tract (Chiu *et al.*, 2021) [3], such as the growth of pathogenic *vibrio* bacteria that can cause disease in shrimp, so the application of probiotic bacteria can directly improve the health and immunity of the shrimp gut (Kumar *et al.*, 2023) [9].

This study showed that the best results in increasing total lactic acid bacteria and decreasing vibrio dominance, were obtained in shrimp experiments fed with the supplementation of probiotics and synbiotics. This is in line with the increased growth and survival of white shrimp as previously described, and indicates that probiotic bacteria given in both treatments (probiotics and synbiotics) have an important effect in increasing the growth and survival of white shrimp during the culture period.

Conclusion

The study concluded that the supplementation of probiotics and synbiotics to the diet can increase the total lactic acid bacteria and reduce the dominance of Vibrio in the intestines of white shrimp, as well as increase the growth and survival of white shrimp (*L. vannamei*).

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