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The combination of *Lactobacillus* sp. and turmeric flour (*Curcuma longa*) in feed on growth, feed conversion and survival ratio of *Litopenaeus vannamei* Boone, 1931

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ABSTRACT

White shrimp (*Litopenaeus vannamei*) cultivation activities have developed very rapidly in Indonesia. White shrimp aquaculture which is carried out intensively with high stocking densities has caused several problems, such as low survival and suboptimal growth. The purpose of this study was to increase growth, feed conversion and survival of white shrimp through a combination of probiotics (*Lactobacillus* sp.) + turmeric flour (*Curcuma longa*) in feed. The method used in this study was Completely Randomized Design (CRD) with four treatment levels and three replications. The results revealed a significant effect ($P<0.05$) on the value of the daily growth rate (DGR), absolute growth rate (AGR), specific growth rate (SGR), survival rate (SR), feed conversion ratio (FCR), and feed efficiency (FE), but no significant effect ($P>0.05$) on the value of the absolute length rate (ALR). Giving probiotics 10 ml+5 g turmeric flour/kg feed (treatment B) was the best treatment for white shrimp aquaculture, including DGR, AGR, ALR, LPS, SR, FCR, and FE values. Furthermore, the results also revealed that with increasing concentration of turmeric flour in the feed, it was negatively correlated to the growth and survival of white shrimp.

Introduction

White shrimp (*Litopenaeus vannamei*) has advantages with high nutritional value and large market demand in domestic and international markets (Mahbubillah, 2011). The contribution of white shrimp to the total world shrimp trade reaches 83% (FAO, 2019), indicating a high demand for this commodity. The production data of the white shrimp in Indonesia in 2019 reached 517,397 tons and is estimated to increase by 250% to reach 1,290,000 tons in 2024 (KKP, 2021). Production continues to increase because white shrimp has high economic value with prices ranging from Rp. 70,000/kg (Mauladani *et al.*, 2020). In addition, white shrimp can live with a wide salinity range, which is between 1-40 ppt (Saoud *et al.*, 2003). Furthermore, white shrimp has other advantages, where it can live in tropical and subtropical climate countries (Burford *et al.*, 2004). This reason is a factor that causes white shrimp to be widely cultivated by aquaculture businessman in Indonesia.

The cultivation technology applied to white shrimp continues to grow rapidly, one of which is the use of probiotics to increase growth, immune system, survival and protein structure of white shrimp (Lin *et al.*, 2004; Gomez and Shen, 2008; Luis-Villsenor *et al.*, 2013). This is done to prevent the shrimp from being attacked by bacterial and viral diseases. In addition, probiotics can improve feed quality so that feed absorption increases so that it is more efficient and effective. Furthermore, other studies have also revealed that turmeric (*Curcuma longa*) also has the function of increasing the immune system in white shrimp (Vanichkul *et al.*, 2010; Lawhavinit *et al.*, 2011; Yu *et al.*, 2018; García-Pérez *et al.*, 2020). Therefore, the combination of probiotics and turmeric has the potential to improve the immune system, growth, digestibility and survival of white shrimp, so it is

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important to do further research on the composition of the appropriate combination.

There are many advantages that can be obtained in the use of probiotic bacteria, especially in the field of aquaculture, including increasing the immune system for the species being cultivated. The interaction of probiotic bacteria either through the aquculture environment or converted through feed is an alternative that can be used to increase shrimp growth performance. Furthermore, various types of natural immunostimulants have been researched and proven effective in increasing the immune system and appetite. Araujo and Leon (2001) stated that one type of natural immunostimulant is turmeric (*C. longa*).

Several studies that have been carried out on white shrimp are increasing immunity and disease resistance using the probiotic *Bacillus subtilis* E20 (Tseng *et al*., 2009; Liu *et al*., 2010), *Bacillus subtilis* E20, a protease-producing probiotic from Natto, was used to boost growth performance (Liu *et al*., 2009), the effect of adding four potential probiotic strains on survival after challenge of immersion with *Vibrio parahaemolyticus* (Balcázar *et al*., 2007), probiotic activity of *Bacillus substilis* UTM 126 against *Vibrio* species in inhibiting and protecting against vibriosis attack on white shrimp larvae (Balcázar and Rojas-Luna, 2007), Effects of *Bacillus* OJ probiotic supplementation and isomaltooligosaccharides in feed on white shrimp gut microbial populations, immunological response, and resistance to white spot syndrome virus (Li *et al*., 2009), *Bacillus* probiotics' capacity to increase water quality and growth in white shrimp during their early development (Nimrat *et al*., 2012) and the use of different concentrations of probiotics on growth (Suwoyo and Mangampa, 2010). While the combination of *Lactobacillus* sp. with turmeric flour on growth, feed conversion and survival of white shrimp has never been done, so it is necessary to study and it is important to do. The role of *Lactobacillus* sp. as a probiotic, it provides health benefits when consumed by optimizing digestive function, while turmeric flour functions as a prebiotic or compound that triggers the growth or activity of beneficial microorganisms (probiotics), stimulates the immune system and increases the absorption of food nutrients in the digestive tract. The results of this study are expected to contribute to practitioners, the farmers and the repertoire of knowledge in the future, especially white shrimp farmers.

Materials and Methods

Location and time

This study was place during April and May of 2021. The research was carried out at the Politeknik Kelautan dan Perikanan Aceh's Teaching Factory for Brackish Water Aquaculture, Indonesia.

Experimental design

A Completely Randomized Design (CRD) with four treatment levels and three replications was used in this study. The factor tested was a combination of *Lactobacillus* probiotics and turmeric flour. Commercial feed with a 34 percent protein content was employed in this study.

Treatment A: Commercial feed (control).
Treatment B: Commercial feed supplemented with probiotics 10 ml+5 g turmeric flour/kg feed.
Treatment C: Commercial feed supplemented with probiotics 10 ml+10 g turmeric flour/kg feed.
Treatment D: Commercial feed supplemented with probiotics 10 ml+15 g turmeric flour/kg feed.

Research procedure

Feed preparation

The probiotics used are commercial probiotics containing *Lactobacillus* bacteria. Provision of probiotics and turmeric flour was given according to the treatment dose. Artificial feed is mixed with probiotics and turmeric flour. Probiotics and turmeric flour were first mixed with 5 g commercial adhesive (Progol) + 100 ml of distilled water. The probiotic and turmeric flour are mixed by spraying on the feed, before the spray bottle is sterilized first. The feed that has been mixed with probiotics and turmeric flour is then dried for 30 minutes.

Container preparation

The container used in this study was a round plastic tank with a diameter of 50 cm, a height of 55 cm and a volume of 100 liters. In addition, a water reservoir is also prepared as a place for water sterilization before being put into the plastic tank. Plastic tank, water reservoirs, aeration hoses, aeration stones and scoops are soaked with 30 ppm chlorine for 30-60 minutes to cleaned from microorganisms (Boyd and Tucker, 2012). The tools that have been soaked are then rinsed using fresh water until they do not smell of chlorine and then dried. The clean plastic tank is filled with 80 liters of water, aeration is installed and left for 24 hours.

Shrimp sample preparation

Juvenile shrimp (size 7.5 g, length 99.85 mm and age 52 days) were taken from CV. Laweung pond,

Pidie Regency, Aceh Province, Indonesia and then sent using plastic packing containing oxygen, then taken to the Teaching Factory for Brackish Water Aquaculture, Politeknik Kelautan dan Perikanan Aceh, Indonesia. The shrimp samples that were still in the plastic packing were put into a tank for acclimatization for three hours. The plastic packing is opened and the shrimp samples are allowed to come out by themselves into the reservoir. The samples was then reared for 8 days in a container to adapt to new environmental conditions. During this adaptation period, the samples were given commercial feed up to a size of 8 – 8.5 g.

Shrimp rearing

The experimental unit used is a plastic tank with a volume of 100 liters of 12 units. The plastic tank is filled with 80 liters of water. The shrimp sample used was juvenile with a size of 8-8.5 g and a density of 12 fish/tank. Shrimp were reared for 28 days. Measurements of length and weight of shrimp were carried out every 7 days. Measurements of length and weight are carried out entirely in each container. Length measurement was carried out using a digital caliper with an accuracy of 0.01 mm and weight measurement using a digital scale with an accuracy of 0.001 gram. Feed was given four times a day at 07.00, 12.00, 17.00 and 21.00 as much as 4 - 3% of the total body weight per day for 28 days. The arrangement of the plastic tank is done randomly.

Water quality during the rearing process is controlled in such a way as to suit the optimum environment for shrimp growth. Once a day, water quality parameters like temperature, dissolved oxygen, salinity, and pH are measured. To maintain water quality, siphoning was carried out every day one hour after feeding.

Research parameters

***Absolute growth rate* (*AGR*)**

The AGR value of shrimp was determined by comparing the initial and end weights. Weight gain of shrimp can be calculated using the formula:

$$AGR = Wt - Wo$$ (Peterson *et al.*, 2012)

Note: AGR is absolute growth rate (g), W_t is final weight (g), W_0 is initial weight (g).

***Daily growth rate* (*DGR*)**

DGR is the average daily weight gain of shrimp and is calculated using the formula:

$$DGR = \left[\frac{(Wt - Wo)}{t}\right]$$ (Muchlisin *et al.*, 2016)

Note: DGR is daily growth rate (g), Wt stands for final weight (g), W0 stands for initial weight (g), and t stands for research time (day).

***Specific growth rate* (SGR)**

The formula for calculating SGR can be found:

$$SGR = \left[\frac{Ln\ Wt\ X\ Ln\ Wo}{t}\right] X\ 100$$ (Muchlisin *et al.*, 2016)

Note: SGR is specific growth rate (%), Wt stands for final weight (g), W0 stands for initial weight (g), and t stands for research time (day).

***Absolute length rate* (*ALR*)**

ALR is calculated by comparing the initial length with the final length. The increase in the length of the shrimp can be calculated using the formula:

$$ALR = Lt - Lo$$ (Peterson *et al.*, 2012)

Note: ALR is absolute length rate (mm), L_t stands for average final length (mm), L_0 stands for average initial length (mm).

***Survival rate* (*SR*)**

The survival rate of shrimp is calculated from the end to the beginning of the experiment. The formula for calculating the survival rate is:

$$SR = \frac{Nt}{No} X\ 100$$ (Ernawati and Rochmady, 2017)

Note: SR is survival rate (%), N_t stands for number of live shrimp at the end of the experiment (ind), N_0 stands for number of live shrimp at the initial of the experiment (ind).

***Feed conversion ratio* (*FCR*)**

The formula for calculating the FCR is to divide the amount of feed given by the total weight growth of shrimp:

$$FCR = \left[\frac{F}{Wt - Wo}\right]$$ (Peterson *et al.*, 2012)

Note: FCR is feed conversion ratio, F stands for weight of the feed given during rearing (g), W_t stands for shrimp biomass at the end of rearing (g), W_0 stands for shrimp biomass at the beginning of rearing (g).

***Feed efficiency* (*FE*)**

FE indicates the level of feed efficiency that can be digested by shrimp. Good feed quality will show a high efficiency value as well. Feed efficiency can be calculated by the formula:

$$FE = \frac{1}{FCR} X\ 100$$ (Peterson *et al.*, 2012)

Where: FE is feed efficiency (%), FCR stands for feed conversion ratio.

Data analysis

The data were analyzed using the Analysis of Variance (ANOVA) with the SPSS version 20. Duncan's test was carried out on treatments that had a significant effect.

Results

The results of the analysis of feeding combined with probiotics + turmeric flour showed a significant effect ($P<0.05$) on the value of DGR, AGR, SGR,

SR, FCR and FE, but had no significant effect (P>0.05) on the ALR value. The highest DGR value was obtained in treatment B reaching an average value of 0.162 ± 0.004 g, followed by treatment C (0.157 ± 0.007 g), treatment D (0.153 ± 0.010 g) and treatment A (0.133 ± 0.004 g). The highest AGR value was obtained in treatment B reaching an average value of 4.527±0.102 g and the lowest in treatment A (3.723±0.104 g). The highest ALR value was obtained in treatment B reaching a mean value of 14.867±0.673 mm and the lowest in treatment D (13.587±0.861 mm). The highest SGR value was obtained in treatment B reaching a mean value of 16.200±0.361% and the lowest in treatment A (13.267±0.376%). The highest SR value was obtained in treatment B reaching a mean value of 93.333±3.333% mm and the lowest was in treatment A (63.333±6.667%). The highest FE value was obtained in treatment B reaching an average value of 56.500±3.071% and the lowest in treatment D (32.730±4.067%). The best FCR value was obtained in treatment B reaching a mean value of 1.780±0.093 and the worst in treatment D (3.200±0.321).

Discussion

Provision of probiotics 10 ml+5 g turmeric flour/kg feed (treatment B) was the best treatment for white shrimp aquaculture, including DGR, AGR, ALR, LPS, SR, FCR, and FE values. Furthermore, the feed that was given probiotics 10 ml+10 g turmeric flour/kg feed (treatment C) also showed positive results and was better than the control feed and probiotic combination of 10 ml+15 g turmeric flour/kg feed (treatment D). In comparison to the control feed, treatment D had no significant effect on any of the research parameters, in fact the FCR and FE parameters showed that the control feed was better than treatment D. These results revealed that with increasing concentration of turmeric flour in the feed, it would be negatively correlated to white shrimp growth and survival.

Several researchers throughout the world have reported on the use of probiotics in white shrimp aquaculture, such as *Bacillus* spp. probiotics' effects on white shrimp growth and survival (Gullian *et al.*, 2004; Balcázar *et al.*, 2007; Wang, 2007; Gómez and Shen, 2008; Tseng *et al.*, 2009; Zhou *et al.*, 2009; Liu *et al.*, 2010; Silva *et al.*, 2012), but with a combination of probiotics 10 ml+5 g turmeric flour/kg feed can further optimize the growth of white shrimp. This research revealed that turmeric flour could increase the role of probiotics in optimizing the growth of white shrimp. This is because turmeric flour is an immunostimulant made up of plant active chemicals that can boost an organism's disease resistance. (Logambal *et al.*, 2000). In addition, turmeric flour is a biocompatible, biodegradable, cost-effective, and environmentally friendly natural immunostimulant (Ortuno *et al.*, 2002).

In addition, the role of probiotic *Lactobacillus* sp. in this study is also very important as a key component in increasing growth, feed efficiency and survival of white shrimp. According to Fuller (1987) and Gram *et al.* (1999), probiotics are live microbial products that serve as feed supplements and benefit the host by enhancing intestinal (microbial) balance. Furthermore, Salminen *et al.* (1999) revealed probiotics bacteria that have a favorable effect on the host body's health. Moriarty (1999) proposed the definition of probiotics in the field of aquaculture as supplementation of living micro-organisms into fish aquaculture containers that directly control biologically the environmental conditions of aquaculture waters. According to Maeda *et al.* (1997) probiotics have physiological and ecological functions to control disease, especially vaccinology.

Table 1. The results of the statistical analysis of the four treatments in the study.

Parameters	Treatments			
	A	B	C	D
DGR (gr)	0.133±0.004^{a}	0.162±0.004^{b}	0.157±0.007^{b}	0.153±0.010^{ab}
AGR (gr)	3.723±0.104^{a}	4.527±0.102^{b}	4.410±0.199^{b}	4.367±0.272^{b}
ALR (mm)	14.047±0.321^{a}	14.867±0.673^{a}	13.700±0.706^{a}	13.587±0.861^{a}
SGR (%)	13.267±0.376^{a}	16.200±0.361^{b}	15.733±0.696^{b}	15.333±0.977^{ab}
SR (%	63.333±6.667^{a}	93.333±3.333^{b}	73.333±6.667^{a}	66.667±6.667^{a}
FCR	3.103±0.279^{b}	1.780±0.093^{a}	2.373±0.047^{a}	3.200±0.321^{b}
FE (%)	35.223±3.751^{a}	56.500±3.071^{b}	38.703±3.013^{a}	32.730±4.067^{a}

Provision of probiotics 10 ml+5 g turmeric flour/kg feed can optimize the survival of white shrimp to reach an average SR of 93.333±3.333%. This result is better than that of Zhou *et al.* (2009) with the highest SR value of white shrimp reaching 85.67 ± 1.15% by testing the probiotic *Bacillus coagulans* SC8168 as an additive in water. Another study also revealed that the feed given the probiotic *B. subtilis* E20 could optimize the mean SR value of white shrimp up to 20% compared to the control diet (Tseng *et al.*, 2009). However, these results were lower than in this study, where the probiotic *Lactobacillus* sp. 10 ml+5 g turmeric flour/kg feed can increase the SR value of white shrimp up to 30% compared to control feed. *Bacillus* probiotics were also used in tiger shrimp (*Penaeus monodon*) and were able to optimize the SR value of shrimp up to 91.68% (Boonthai *et al.*, 2011), but this result was relatively lower than in this study. According to Farzanfar (2006) probiotics are very important in shrimp aquaculture to increase the amount of production and higher quality.

Conclusion

The combination of probiotics and turmeric flour in feed had a significant effect ($P<0.05$) on DGR, AGR, SGR, SR, FCR, and FE values, but had no significant effect ($P>0.05$) on the ALR value. The combination of probiotics 10 ml+5 g turmeric flour/kg feed (treatment B) was the best treatment for white shrimp aquaculture, including DGR, AGR, ALR, LPS, SR, FCR, and FE values. Furthermore, the results also revealed that with increasing concentration of turmeric flour in the feed, it would be negatively correlated to the growth and survival of white shrimp.

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