Administration of probiotic-supplemented diet to improve the growth of carp juvenile (*Cyprinus carpio* L)

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Abstract

This research was conducted to examine the effect of probiotic-added pellet on growth of carp juveniles. Fish having mean weight 4.29±0.16 g was purchased from fish farmers in Tatelu Village, North Minahasa Regency, North Sulawesi Province. After adaptation for one week, the fish were fed pellet supplemented with probiotic at different concentration, including $1x10^6$, $1x10^7$, $1x10^8$, $1x10^9$ cfu.mL^{-1}. The control pellet was not added with probiotics but coated with 2% yellow egg as in the treatment pellets. Fish were culture for four weeks with a feeding rate 5% /body weight/day, and feeding frequency of twice a day at 08.00 and 16.00. The weight of fish was weighed at two and four weeks after feeding. The result showed that final weight, absolute growth and average daily growth were significantly higher in fish fed probiotic pellet compared to control fish.

Keywords: probiotic, aquaculture, gut micro flora, feed efficiency, digestive enzymes

INTRODUCTION

Aquaculture is an effort to cultivate aquatic organisms with human intervention in the cultivation process to increase production and profit. In this case, aquaculture contributes to the production of food, raw materials for the aquaculture and pharmaceutical industries, ornamental fish and restocking fish (Cruz *et al*., 2012). The rapid development of aquaculture along with the increasing demand for fish has encouraged aquaculture to be carried out more intensively (Reverter, 2014). On the other hand, intensification has increased stress levels in fish, increased the risk of disease outbreaks, damaged water quality and economic losses to the aquaculture industry (Fečkaninová *et al*., 2017; Wang *et al*., 2008).

Prevention and treatment of disease in aquaculture generally uses antibiotics and chemicals. However, the use of antibiotics in fish and shrimp farming has caused various problems such as the emergence of antibiotic-resistant bacterial and the accumulation of antibiotic residues in body tissues (Kong *et al*., 2020, Wang *et al*., 2018; Zokaeifar *et al*., 2012). In addition, the intensive and repeated use of antibiotics or chemicals can cause various negative effects such as bioaccumulation, pollution, damage to environmental microbes and suppress the immune system of fish. (Wang & Xu, 2014; Biswas *et al*., 2012; Kartik *et al*., 2014). Citarasu (2010) stated that although antibiotics and chemicals can have a positive effect on fish and shrimp, their use cannot be recommended because of the residues that accumulate in fish and shrimp meat. Therefore, research on the use of feed with ingredients that can improve health status and stimulate the growth of cultivated species is needed in an effort to develop environmentally friendly aquaculture (Hoseinifar *et al*., 2015). One of the ingredients that have the potential and is increasingly being applied is probiotics.

Probiotics recently have been widely applied in aquaculture as an alternative to antibiotics in preventing disease. Probiotics are living micro-organisms that can improve host health through the provision of nutrients and protection against pathogens and can be used as a substitute for antibiotics/chemicals (Perez-Sanchez, 2014; Hoseinifar *et al*., 2018) Probiotics can be in the form of live or dead organisms or microalgae or yeast that are given through feed or into pond water to improve growth performance and disease resistance (Banu *et al*., 2020; Prabhurajeshwar & Candrakanth, 2019).

Research reports showed that the use of probiotics in aquaculture could improve growth, feed digestibility, feed efficiency, appetite, immune system, and fish resistance to pathogens (Hoseinifar *et al*., 2018; Ling *et al*., 2018; Lin *et al*., 2019; Kong *et al*., 2020; Kavita *et al*., 2018). In addition, probiotics could inhibit the development of pathogens, maintain the micro flora balance of the digestive tract, improve digestive function and improve water quality (Wang *et al*., 2018; Sornplang & Piyadeatsoontorn, 2016). Hai (2015) stated probiotics played an important role in providing growth factors and producing digestive enzymes such as proteases, lipases and carbohydrolases. The current study was conducted to examine the effect of *Lactobacillus* sp. added into feed on the growth of carp juvenile. The research was conducted from January – April 2025.

RESEARCH METHOD

Experimental Fish

About 250 healthy carp juveniles with an average weight of 4.29±0.16 g was purchased from fish farmers in Tatelu Village, North Minahasa Regency, North Sulawesi Province.

Probiotics and Feed Preparation

Probiotic bacteria (*Lactobacillus* sp.) was obtained from stock available at the Laboratory of Fish Health, Environment and Toxicology, Faculty of Fisheries and Marine Science. Bacteria were cultured and propagated using MRS agar.

The feed used was commercial pellets having the composition of 31% protein, 5% lipid, 5% crude fibre, 13% ash, and 12% water. The probiotics used as treatment contained $1x10^6$, $1x10^7$, $1x10^8$, $1x10^9$ cfu.mL^{-1}. Probiotic density was prepared by suspending bacterial colonies into 0.85 NaCl and comparing it with McFarland's standard solution to obtain a density of $1x10^9$ cfu.mL^{-1}. Furthermore, the concentration of bacteria was diluted by mixing 1 mL of solution containing $1x10^9$ cfu.mL^{-1} into 9 mL NaCl to obtain a concentration of $1x10^8$ cfu.mL^{-1}, and so on until $1x10^6$ cfu.mL^{-1} was obtained. Each treatment was added into the pellet by spraying evenly then dried. After drying, the pellets were coated with egg yolk as much as 2%, and then dried again. The control diet was not added with probiotics but coated with 2% egg yolk too. The treatment feed was then put in a plastic box and stored in the refrigerator until used.

Research Procedure

The fish obtained were spread into five 60 L-buckets with a density of 50 individuals each. Before starting the experiment, the fish were adapted for one week. Fish were given standard feed without the addition of probiotics as much as 5% per body weight per day, and given twice per day at 08.00 and 16.00. Each bucket was aerated continuously using an aerator. A water pump (capacity 1500 L/H) was also provided for water recirculation.

After adaptation, the density was set to 25 fish/bucket. The fish were fed pellet already supplemented with probiotic. The feed dosage was the same as in the adaptation period, namely 5%/body weight/day, twice a day at 08.00 and 16.00. To maintain good water quality, 30% of the water volume was replaced every 2 days. Syphoning was also carried out every day to remove uneaten pellet and fish waste.

Fish growth

The final weight was weighed at two and four weeks after feeding. Absolute growth and average daily growth were calculated following the formula used by Kong et al. (2020) and Abdel-Ghany *et al*. (2020):

Absolute growth, (g) = Final weight - initial weight

$$\text{Average daily growth,} \quad \text{ADG } (\%) = \frac{\ln(\text{final weight}) - \ln(\text{initial weight})}{\text{feeding duration}} \; x \; 100$$

RESULT AND DISCUSSION

Final Weight of Fish

The addition of probiotics into feed can stimulate fish growth. After being given probiotic feed for two weeks, the final weight of fish (initial weight 4.29 ± 0.16 g) at all treatment did not display any differences (p>0.05). But after four weeks of feeding, the final weight at treatments C and D was significantly heavier than that of control fish (p <0.01). The largest final weight was achieved in fish in treatment D then in treatment C while the smallest was observed in treatment A (without probiotics) (Figure 1).

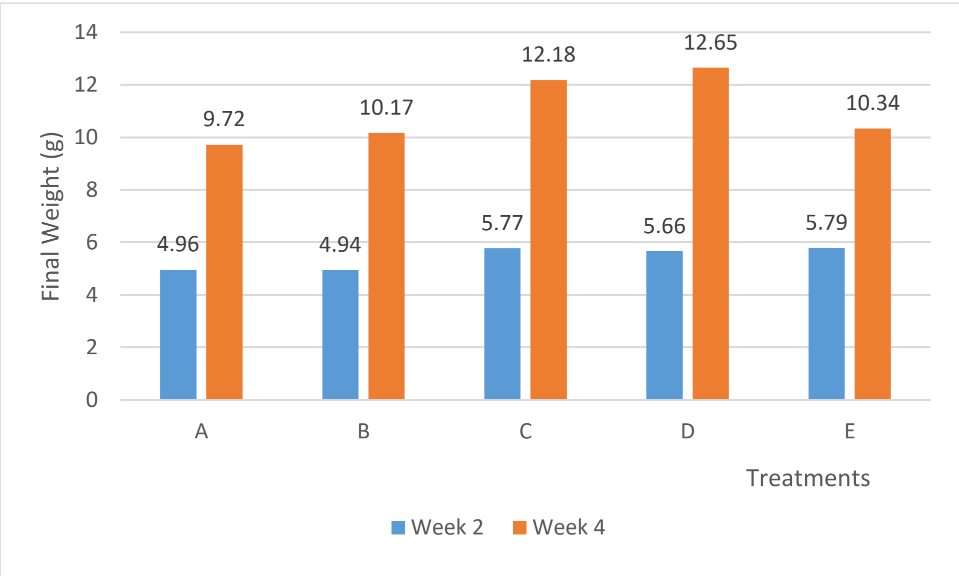

Figure 1. Final weight of fish weighed at two and four weeks after feeding probiotic-added pellet (A = Control pellet; B = probiotic 1x10^6 cfu.mL^{-1}, C = probiotic 1x10^7 cfu.mL^{-1}, D = probiotic 1x10^8 cfu.mL^{-1}, E = probiotic 1x10^9 cfu.mL^{-1})

Absolute and Daily Growth

Feeding with the addition of probiotics at different concentrations for two weeks did not influenced fish growth ($p>0.05$). The growth both absolute and daily growth were significantly affected after feeding for four weeks ($p <0.01$). The best absolute growth and average daily growth rate were achieved in fish in treatment D and C. In these two treatments, the growth of fish was significantly larger compared to control fish and to all other treatments, but no significantly difference was observed between the two. The absolute growth of fish in treatment D and C reached 53.95% and 45.30% greater than control fish (Table 1).

Table 1**.** Absolute and daily growth of fish fed probiotic-added pellet

Treatment	Absolute growth (g)	Average daily growth (%)
A	5.43±0,34^{a}	2.92±0,12^{a}
B	5,88±0,49^{ab}	3.07±0,17^{ab}
C	7.89±0,52^{c}	3.72±0,15^{c}
D	8.36±0,42^{c}	3,86±0,12^{c}
E	6,05±0,37^{b}	3,14±0,13^{b}

Means with different superscript in the same column were significantly different.
(A = Control feed; B = probiotic 1x10^6 cfu.mL^{-1}, C = probiotic 1x10^7 cfu.mL^{-1}, D = probiotic 1x10^8 cfu.mL^{-1}, E = probiotic 1x10^9 cfu.mL^{-1})

The result of this study was consistent with our previous study in which probiotic candidates originated from the intestines of carp considerably improved growth performance and feed efficiency after feeding for 30 days. In that study, the best growth was obtained in fish (mean initial weight 2.09 ± 0.27 g) fed for 30 days with pellet added with probiotic at 1x10^7 - 1x10^8 cfu.mL^{-1} (Manoppo *et al*., 2019). So, probiotics derived from carp as a host (host derived probiotic) can be used to stimulate the growth of the same species. Kong *et al*. (2020) had also reported that *Channa argus* (average weight 9.50 ± 0.03 g) fed pellet added with probiotic *L. lactis* isolated from the intestine of *C. argus* at a concentration of 1.0 × 10^8 cfu /g pellets for 56 days significantly increased the final fish weight, weight gain, feed efficiency ratio, specific growth and protein efficiency ratio of that species.

Probiotic is beneficial for promoting growth of aquatic organisms. The results of a study conducted by Manoppo *et al*. (2019) showed that the addition of probiotics isolated from the intestines of catfish at 1 x 10^8 cfu. mL^{-1} was able to improve weight gain, daily growth, feed efficiency of carp and reduce FCR after being given for 21 days. Report by Pangalila *et al*. (2020) showed *Lactobacillus* sp. isolated from the intestines of catfish (*Clarias* sp.) significantly increased the best phagocytic activity and leukocyte counts were achieved in fish fed with additional probiotics 1x10^8 CFU/mL. Chowdhury & Roy (2020) reported that striped catfish (*Pangasianodon hypophthalmus*) fed with the addition of probiotics 0.20% / kg of feed had higher weight gain, relative growth rate, and protein efficiency and survival ratio compared to control fish ($p<0.05$). The feed conversion ratio also significantly decreased ($p <0.05$) compared to control fish and other treatments.

In tilapia fish, application of probiotics-added feed with a 7-day cycle alternating with feed without probiotics was effective in increasing the growth of fish (Tachibanaa *et al*., 2020). Abdel-Ghany *et al*. (2020) also reported that feeding with the addition of probiotics to tilapia for 56 days increased the growth and utilization of feed and modulate the composition of bacteria in the fish gut. Tan *et al*. (2019) reported the addition of the probiotic *Rummeiliibacillus stabekisii* in feed and given for 8 weeks significantly increased weight gain, Feed conversion ratio and feed efficiency. In a study conducted by Mohammadian *et al*. (2019), carp with an average size of 65 g treated with *L. casei* probiotics for 60 days had higher growth and feed intake than control fish due to the increased production of protease, amylase, and cellulose digestive enzymes. Nguyen *et al*. (2017) found that olive flounder *Paralichtys olivaceus* fed diet supplemented with *L. lactis* displayed better growth indicated by the increase in daily growth rate and decrease feed conversion ratio. Similar result was reported by Jang *et al*. (2019) in which olive flounder (*P. olivaceus*) treated with *L. plantarun* for 8 weeks had better growth than control fish. Ullah *et al*. (2018) found that Mori fish (*Cirrhinus mrigala*) fed with the addition of probiotics for 90 days had a higher final weight, total length, weight gain, and daily growth compared to control fish ($p <0.05$). Digestive enzymes, namely cellulose, protease, and amylase were significantly higher in fish given probiotics compared to control fish.

Supplementation of probiotic in shrimp culture was also reported. Xie et al. (2019) found that white shrimp treated with probiotics as much as 2 g / kg of feed for 8 weeks had a higher daily growth rate and weight gain compared to shrimp that were not treated with probiotics. Another report showed that combination between *L. pentosus, L. fermentum, Bacillus subtilis*, and *Saccharomyces cerevisiae* significantly improved shrimp growth and health status with the best results achieved at 10^7-10^9 cfu / kg of feed (Wang *et al*., 2019). Based on the results of meta-analysis conducted by Toledo *et al*. (2019), the addition of probiotics in shrimp feed could increase specific growth rate, feed conversion ratio and shrimp survival.

According to Tachibanaa *et al*. (2020), probiotics given to fish can increase growth by regulating the micro flora balance in the digestive tract and strengthening the immune system. Micro flora are very important in producing digestive enzymes such as proteolytic, amylolytic, cellulolytic, lipolytic which are needed to digest proteins, carbohydrates, cellulose, and lipids. Pangalila *et al*. (2022) found that probiotic had strong antagonistic activity against *A. hydrophilla* which was indicated by the presence of a clear zone with a diameter of 26.88 mm around the probiotic. Asaduzzaman *et al*. (2018) had also reported that the probiotics fed to Malaysian Mashseer (*Tor tambroides*) drastically regulated the microbiota composition in the gut where the number of proteolytic, cellulolytic and lipolytic bacteria was significantly increased in fish given probiotics compared to control fish. The increase in digestive microflora will contribute to metabolism and feed utilization which in turn results in increased growth.

Conclusions

Oral administration of probiotic originated from carp intestine was potential to improve fish growth. The addition of probiotic at $1x10^7$ $1x10^8$ $cfu.mL^{-1}$ demonstrated the best growth response.

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