

The use of solid probiotics in feed to growth and survival rate of *mantap* common carp (*Cyprinus carpio*)

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Abstract. This study aims to determine the effect of solid probiotics used in feed on the growth and survival rate of *mantap* common carp (*Cyprinus carpio*). This research was conducted in the Ciparanje Hatchery, Faculty of Fisheries and Marine Sciences, Universitas Padjadjaran. The study was conducted for 60 days from July to September 2018. The research method used was experimental method using Completely Randomized Design (CRD) consisting of 4 treatments and 4 replications, solid probiotics given with treatment A (0 g kg⁻¹), treatment B (0.75 g kg⁻¹), treatment C (1.5 g kg⁻¹) and treatment D (2.25 g kg⁻¹). The parameters observed were average daily gain (ADG), feed efficiency (FE), protease enzyme activity in the digestive tract, survival rate (SR) and water quality which included dissolved oxygen (DO), temperature and pH. Data were analyzed using analysis of variance with F test with 95% confidence level and the difference between treatments was tested by Duncan multiple-range test, then protease enzyme activity and water quality were analyzed descriptively by comparing to related literature and water quality national Indonesian standard. The results showed that the use of probiotics in feed had an effect on the growth of *mantap* common carp. The addition of probiotics at a dose of 1.5 g kg⁻¹ of feed in treatment gave the optimal yield with the daily growth rate of 2.01%, feed efficiency 38.63%, protease enzyme activity 139.20 unit, and survival rate 83.75%.

Key Words: *mantap* common carp, average daily gain, probiotic, feed efficiency, survival rate.

Introduction. Common carp (*Cyprinus carpio*) is a consumption freshwater fish which well develops in Indonesia. The demand for fresh common carp product is relatively high, making the common carp one of the favorite fishes to the Indonesians. The demand has never declined and even increases from year to year. Common carp production in Indonesia was up to 340,863 tons (Ministry of Maritime Affairs and Fisheries 2014) in 2013, and it was targeted to 723,500 tons in 2018.

The success of common carp farming cannot be separated from some factors. Feed is one important factor in farming that influences fish growth rate. However, the problem is that fish has limitation in digesting feed. Fish nutrition has advanced dramatically in recent years with the development of new, balanced commercial diets that promote optimal fish growth and health (Craig et al 2017).

One effort to improve feed quality is to add probiotic into feed to improve fish growth (Perez-Sanchez et al 2014). The use of probiotics as feed enrichment has now become one of the efforts to maintain eco-friendly aquaculture (Carnevali et al 2017). Among them is a definition considered appropriate for aquaculture which is described as "any microbial cell provided via the diet or rearing water that benefits the host fish, fish farmer or fish consumer, which is achieved, in part at least, by improving the microbial balance of the fish" (Ige 2013). Adding probiotic into feed may be a solution to improve feed quality. Mohammadi et al (2016) said that there was no significant difference in the

effect of the dietary yeast on survival rate (%SR) and condition factor (CF), but a significant increase in the protein content of body was observed by addition of probiotic in the diet in *Cichlasoma trimaculatum*. According to Dhingra (1993) in Arief et al (2014), probiotic is useful to regulate microbial environment in intestine, prevent pathogenic intestinal microorganism and improve feed efficiency by releasing enzyme to help feed digestion process. The existence of dry probiotic is currently a choice for its practicality, but with unknown dosage. This study aims to determine the effect of solid probiotics used in feed on the growth and survival rate of *mantap* common carp (*Cyprinus carpio*).

Material and Method. The research was conducted in July-September 2018 with 60 days of farming period. The farming activities of *mantap* common carp were conducted at the Ciparanje Hatchery Zone, Faculty of Fisheries and Marine Science, Padjadjaran University. The activity of protease enzyme in fish digestion was examined at the Bioinformatics and Molecular Biotechnology Research Center Laboratory, Padjadjaran University.

The devices used in this research were: pH meter (Lutron/WA-2017SD), dissolved oxygen (DO) meter (Lutron/WA-2017SD), digital scale (UTE), 17 aquaria (0.4 m x 0.6 m x 0.4 m), aerator set, scoop net, plastic bags, camera, label sticker, water heater, measuring glass cup, basin, stationary, plastic hose, sprayer. Twenty juveniles of common carp were placed into each aquarium and acclimated for 3 days. pH, DO, temperature and water level were controlled during experiment.

The materials used in this research are: commercial solid stock probiotic containing *Latobacillus* sp. and *Bacillus* sp., commercial feed containing 30.39% of protein, 2.03% lipid, 13.36% ash, 5.51% crude fiber, 4.81% weight extract without nitrogen, *mantap* common carp of 5-7 cm in total length obtained from Balai Benih Ikan Ciparay, and molasses.

The research method used was experimental method using Completely Randomized Design (CRD) consisting of 4 treatments and 4 replications. The amount of solid probiotics given were as follows: treatment A (0 g kg⁻¹), treatment B (0.75 g kg⁻¹), treatment C (1.5 g kg⁻¹) and treatment D (2.25 g kg⁻¹). The parameters observed were average daily gain (ADG), feed efficiency (FE), protease enzyme activity in the digestive tract, survival rate (SR) and water quality which included DO, temperature and pH.

Procedure. The research is conducted through some phases, such as preparing farming media, test fish, making of test feed, 60 days of raising and enzyme activity in intestine test. The test fishes are fed three times daily with 5% of their weight. A shift pond is performed once daily, while the test fish sampling is performed once every ten days and temperature, DO and pH measurements are performed once in 7 days.

The parameters observed include ADG, feed efficiency, protease enzyme activity in digestive tract, survival and water quality parameters including temperature, pH and DO. According to Effendie (1997), the formula for ADG calculation is:

$$ADG = \frac{W_t - W_0}{t} \times 100$$

where: ADG = average daily gain;

W_0 = weight of fish biomass d-0 (g);

W_t = weight of fish biomass d-t (g);

t = research period (day).

FE was calculated according to Tacon (1987):

$$FE = \frac{W_t - W_0}{f} \times 100$$

where: FE = feed efficiency (%);

W_0 = weight of fish biomass d-0 (g);

W_t = weight of fish biomass d-t (g);

f = feed consumed during research (g).

Activity was calculated according to Kaiser et al (1996):

$$\text{Activity} = \frac{AS - AB}{0.001 \times t \times v}$$

where: Activity = enzyme activity (unit);
 AS = absorbance sample;
 AB = absorbance template;
 v = enzyme volume (mL)
 t = time of hidrolisis (minutes).

Survival rate was calculated according to Effendie (1997):

$$SR = \frac{N_t}{N_0} \times 100$$

where: SR = fish survival rate during research (%);
 N_t = number of fish at T_t (tail);
 N₀ = number of fish at T₀ (tail).

Data analysis. Data were evaluated using analysis of variance with F test with 95% confidence level and the difference between treatments was tested by Duncan multiple-range test, then protease enzyme activity and water quality were analyzed descriptively by comparing to related literature and water quality national standard according to SNI (1999).

Result and Discussion

Fish weight increase. Based on the research results, it was found that fish weight will increase in line with the raising period. This shows that probiotic provision may enhance the nutritional utilization by fishes. This fish weight growth with probiotic treatment shows that digestive enzyme plays a role which makes the fishes well digest their feed. The enzyme expected to play the role in digestion is protease and amylase (Widanarni et al 2012). Previous studies indicated that protease is capable of decreasing the effect of trypsin inhibitors improving protein digestibility and feed efficiency (Yigit et al 2018). Amylase is a digestive enzyme that aids in the breakdown of carbohydrates by breaking the bonds between sugar molecules in polysaccharides through a hydrolysis reaction. It is important in the digestion of starch into sugars to make available energy sources for the body (Bhilave et al 2014).

Generally the addition of probiotics increases growth higher than without probiotics. Meanwhile from the Figure 1, it can be seen that the highest increase in growth was treatment D with 2.25 g kg⁻¹ probiotics. Probiotic bacteria are factors that play a role in maintaining the normal growth and function of aquatic animals by working as a source of nutrients, vitamins and digestive enzymes, which have good effects on the use of feed, nutrient absorption and increased growth (Dawood et al 2014; Aragona et al 2017; Nath et al 2019).

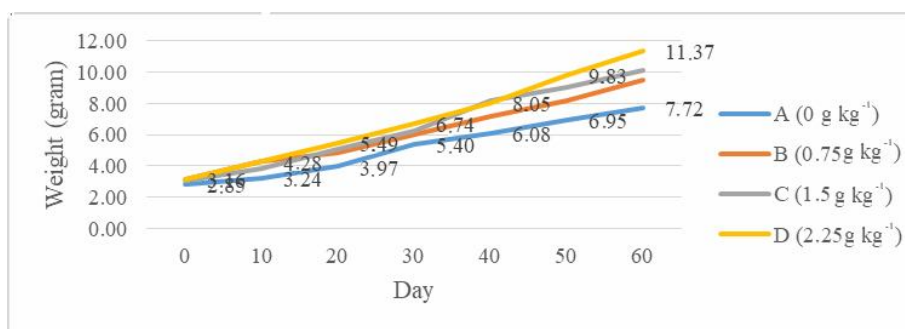


Figure 1. Fish weight increase of *mantap* common carp in every treatment.

Average daily gain. The ANOVA test shows that the treatment given results significantly different outcomes at confidence level of 95% ($p < 0.05$) in *mantap* common carp's ADG. The next test with Duncan's Multiple Test shows that the probiotic treatment with different dosages results in significantly different outcomes between treatments.

Figure 2 shows *mantap* common carp's value for each treatment during the 60 days of raising period. Treatment C with probiotic provision of 1.5 g kg^{-1} feed is the optimal treatment with 2.01% AVG. While the addition of 0 g kg^{-1} and 0.75 g kg^{-1} probiotic showed a less ADG results compared to 1.5 g kg^{-1} or 2.25 g kg^{-1} probiotic addition. Bacteria in the digestive tract have important and varied enzymatic potentials, and it seems logical to think that the enzymatic mass that is lodged in the digestive tract can affect all or most of the metabolism of host animals (Bairagi et al 2002). The amount of these secreted enzymes also increases pursuant to the amount of probiotic dosage given and, eventually, the digested amount of feed also increases. Increased digesting capacity also means higher nutrient available for absorption by the body, and thus the growth also increases (Setiawati et al 2013).

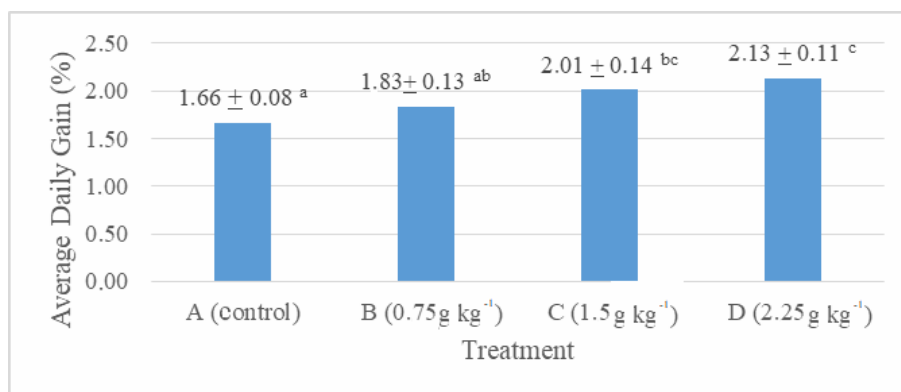


Figure 2. Average daily gain of *mantap* common carp in every treatment (different superscript letters show significantly different effects).

According to Lamarre et al (2007) catabolism of food is organized to efficiently harness the chemical energy of substrates for use in anabolic and other life-sustaining processes. During early life, the failure to meet nutritional needs because of inefficient or poorly developed ingestion, digestion, or assimilation processes will result in impaired growth, starvation, reduced viability, and ultimately death (Blier et al 1997). Thus, it has been suggested that complete functionality of the digestive organs (i.e., the availability of digestive enzymes and of key metabolic enzymes) could set substantial physiological limitations on the growth and (or) survival of juveniles (Lammare et al 2007). Accordingly, fast relative growth of the digestive organs was observed during early life in *Dentex dentex*, indicating the priority of the development of these organs for growth and survival (Sala & Esparza 2005); this is also available for the *mantap* common carp in this research.

Feed efficiency. Improving feed efficiency (FE) is a key to reducing production costs in aquaculture and to achieve sustainability for the aquaculture industry. Feed costs account for 30-70% of total production costs in aquaculture (de Verdal et al 2018). The ANOVA test shows that the treatment given results in significantly different outcomes at confidence level of 95% ($p < 0.05$) in the test *mantap* common carp's feed efficiency. The next test using Duncan's Multiple Test shows that the treatment of probiotic provision with different dosages results in significantly different outcomes.

Figure 3 shows the feed efficiency value of *mantap* common carp for each treatment during the 60 days of raising period. Treatment C with probiotic provision of 1.5 g kg^{-1} feed is the optimal treatment with 38.63% feed efficiency. The feed efficiency with probiotic addition is higher than that without probiotic addition into feed, showing the role the probiotic bacteria play in digestive tract. According to Ahmadi et al (2012), the proportion of probiotic bacteria colony in feed makes the probiotic bacteria work

maximally in fishes' digestion, thus the fishes' digestive capacity gets higher in absorbing food essence.

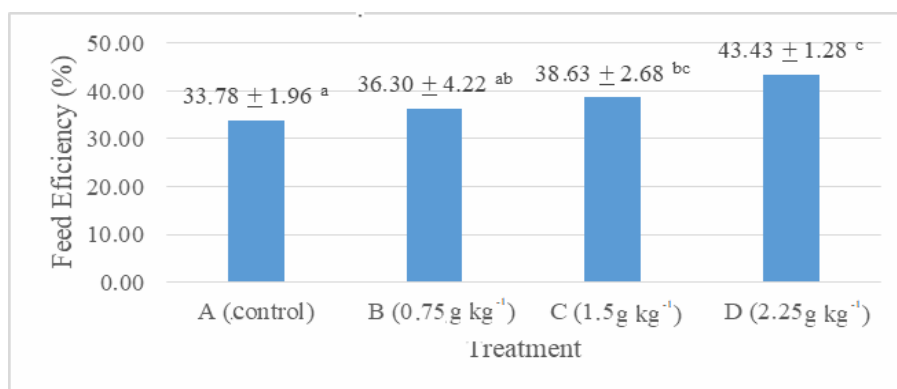


Figure 3. Feed efficiency of *mantap* common carp in every treatment (different superscript letters show significantly different effects).

Feed efficiency can be defined as pounds of fish gain produced per pound of dry matter (DM) consumed (Hutjens 2004). In fish, measuring FE implies measuring feed intake, which is highly complex as fish are generally reared in water and in large groups (De Verdal et al 2018). Fish industries have used FE (feed to gain or gain to feed ratio) as a benchmark for profitability (Pramono 2018). The "new focus" on maximizing efficiency reflects the fact that as fish consume more feed, digestive efficiency decreases because the relationship between net energy intake and gain production is subject to diminishing returns (De Verdal et al 2018). The "traditional focus" was that as fish consume more feed to support higher fish gain production, the proportion of digested nutrients captured as fish gain is proportionally higher. Treatments C and D are more efficient compared to other experimental treatments.

Protease enzyme activity. The enzyme test in digestive tract was conducted with two treatments: control and probiotic addition. Based on data of Figure 4, the protease enzyme activity value in *mantap* common carp's digestive tract with probiotic treatment is higher than that of control. The mean value of protease enzyme activity with the control and the probiotic addition is respectively 107.14 and 139 units. The reason the enzyme activity value of probiotic treatment is higher is expected due to probiotic activity in the fishes' digestive tract. According to Suciati et al (2016), *Lactobaccillus* sp. has continuously increasing proteolytic activity up to day 7 in MRS media. That the enzyme activity with probiotic treatment is higher than that of the control may indicate that probiotic mixed in the feed actually enters fishes' digestive tract. Salminen & Atte (2004) in Andriani et al (2017) state that bacteria may be classified as probiotic bacteria when they remain active (alive) in gastric acid condition. Bacteria at growth phase produce primary metabolite in the form of extracellular protease enzyme. Probiotic bacteria produce extracellular protease enzyme naturally to hydrolyze polypeptides to peptides and amino acids (Wilson & Remigio 2012).

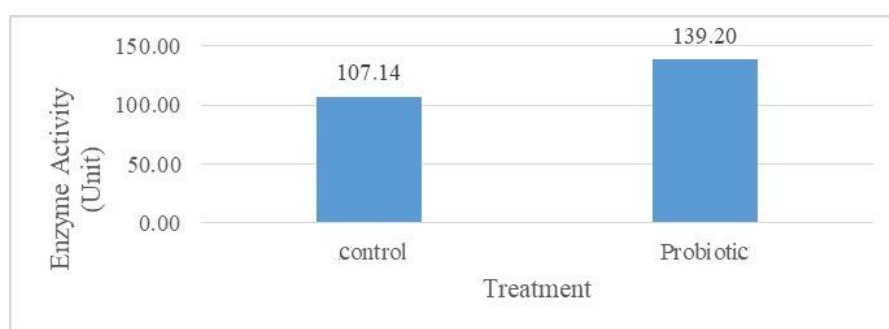


Figure 4. Enzyme activity in digestive tract with probiotic treatment.

The results of ANOVA test with the survival data of *mantap* common carp show that the treatment given is not significantly different at confidence level of 95% ($p < 0.05$). The next test using Duncan's Multiple Test shows that the probiotic addition with different dosage treatment results in not significantly different outcomes. The survival of *mantap* common carp remains good. The highest survival is obtained with treatment D (2.25 g kg⁻¹ feed) of averagely 85%. Balcazar et al (2006) had demonstrated that the intestinal microbiota has a strong effect on the health status of animals.

Probiotics are usually members of the healthy intestinal microbiota; therefore, they may provide an alternative way to reduce the use of antibiotics in aquaculture, since their addition can assist in returning a disturbed microbiota to its normal beneficial composition. Probiotic addition will directly increase the effectiveness of intestinal microbes and eventually increase growth (Widjastuti et al 2017). The capability of advantageous microbes in inhibiting pathogenic microbes' growth shows their capability to maintain the microfloral balance in fishes' digestive tract. This ability is related to their capability to produce antimicrobial substances like peptides which are synthesized in ribosomes (Setiawati et al 2013). Water quality plays an important role in support of *mantap* common carp's life and growth. The results of observation of some parameters of water quality, which are temperature, pH and DO in all treatments during the research are presented in Table 1. The water quality will certainly affect fishes' health. The bad quality of water in the rearing medium may lead to low growth rate, survival and increase the amount of disadvantageous bacteria. The quality of water in the rearing medium is still appropriate for fish growth and survival, but the DO is still below the standard and non-optimal. Based on SNI (Indonesian National Standardization) number SNI: 01- 6137 – 1999 the minimum DO for common carp farming is 5 mg L⁻¹ (SNI 1999).

Table 1
Water quality (temperature, pH and DO) in all treatments during the research

Treatment	pH	Temperature (°C)	DO (mg L ⁻¹)
A (0 g kg ⁻¹)	7.15-7.45	24.7-27.4	4.2-5.6
B (0.75 g kg ⁻¹)	7.11-7.52	24.5-28.3	4.1-5.9
C (1.5 g kg ⁻¹)	7.18-7.6	25.5-28.2	4.3-5.9
D (2.25 g kg ⁻¹)	7.08-7.58	25-28.6	4.6-5.6
SNI (1999)	6.5-8.5	25-30	> 5

Conclusions. From the result we can conclude:

- solid probiotic in fish feed can increase average daily gain (ADG) and feed efficiency (FE);
- the highest average daily gain of *mantap* common carp in this research is obtained at treatment 1.5 g kg⁻¹ by 2.01%;
- treatment C with probiotic provision of 1.5 g kg⁻¹ feed is the optimal treatment with 38.63% feed efficiency;
- the highest survival is obtained with treatment D (2.25 g kg⁻¹ feed) of averagely 85%.

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