Growth and feed efficiency enhancement by probiotic originating from intestine of carp, *Cyprinus carpio*

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Abstract. The objectives of this study were to evaluate the effects of probiotic isolated from digestive tracks of carp on growth and feed efficiency. Carp fingerlings with an average weight of 2.09±0.27 g were gathered from Tatelu Freshwater Aquaculture Fisheries Center. The probiotic *Lactobacillus* sp. was previously isolated from carp intestine at Fish Health, Environment and Toxicology Laboratory of the Fisheries and Marine Science Faculty Sam Ratulangi University Manado, Indonesia. After purification, probiotic was subsequently mixed into feed. The density of probiotic bacteria added to the feed was $1x10^6$ cfu mL^{-1}, $1x10^7$ cfu mL^{-1}, $1x10^8$ cfu mL^{-1}, $1x10^9$ cfu mL^{-1}. Commercial feed without addition of probiotic was used as a control treatment. Probiotic-supplemented feed was fed to carp fingerling having an average weight of 2.09±0.27 g for 30 days at a dose of 5%/body weight/day, twice a day at 09.00 am and 16.00 pm. Data collected included fish weight, feed efficiency and feed conversion ratio measured at the end of the study. The results found the addition of probiotic bacteria into feed significantly affected weight gain, daily growth, feed efficiency, feed conversion ratio ($p<0.01$) with the best results obtained in fish fed probiotic pellet supplemented with $1x10^8$ cfu mL^{-1}. Thus, incorporation of probiotic bacteria isolated from carp intestines is able to enhance growth, feed efficiency as indicated by the increase in growth, feed efficiency and reduce food conversion ratio.

Key Words: food conversion ratio, friendly aquaculture, growth promoter, immunomodulator.

Introduction. Carp (*Cyprinus carpio* L.) is a freshwater fish species widely cultivated in North Sulawesi. Intensification of aquaculture in limited land with high density has caused problems especially the declining quality of the environment due to high organic matter discharge, stress on fish, poor growth, and increased disease events which result in a significant decrease in production (Manoppo et al 2016; Hoseinifar et al 2015).

Antibiotics and chemicals have long been used to prevent or treat diseases in cultured fish. This has resulted in the emergence of antibiotic resistant pathogens in aquaculture environments and accumulation of residues in aquatic products (Tan et al 2019). Ullah et al (2018) also stated that antibiotics have been proven to have harmful effects on the environment and health of fish and have been banned in many countries. In addition, disease control through the use of antibiotics/chemicals had created problems such as bioaccumulation, pollution, and suppression of the fish's immune system (Biswas et al 2012; Babu et al 2013; Wu et al 2013). Sornplang & Sudthidol (2016) reported food of animal origin has been found to contain antibiotic residues in meat due to excessive use of antibiotics and if the meat is consumed by humans then the residue will be accumulated in the human body causing resistance to drugs.

At present, the use of herbs, immunostimulants, bioflocs and probiotics has attracted much attention to be used as an alternative to antibiotics and chemicals (Nguyen et al 2019; Adipu et al 2019; Dawood & Koshio 2016; Kuhn et al 2010; Crab et al 2012; Burrels et al 2001; Payung et al 2017). The main challenge of fish and shrimp farmers is to increase growth and at the same time reduce the incidence of disease. The application of probiotics is a safe alternative to antibiotics and has been used well by

farmers as feed additives and biological control agents (Ullah et al 2018; Wang et al 2018; de La Banda et al 2010; Wang et al 2008). FAO defines probiotics as living microorganisms which if given in appropriate quantities can improve fish health (FAO 2002). Prabhurajeshwar & Chandrakanth (2019) stated that probiotics are living organisms that can improve health if added to feed. Probiotic bacteria not only function as biocontrol for fish diseases, but also as an activator to increase the nutritional value of feed (Kumar et al 2013; Ullah et al 2018). Probiotics produce protease, amylase and lipase enzymes, as well as growth factors such as vitamins, fatty acids and amino acids so that absorption of nutrients becomes more efficient. Probiotics also produce antimicrobial ingredients so that they can kill or inhibit pathogens, increase growth, function as immunostimulants that can enhance the fish's immune system against disease, and regulate digestive microbial balance (Wang et al 2019). So the use of probiotics is a good management strategy to increase growth to control pathogens in fish and crustaceans.

Currently there are several commercial probiotics derived from several bacterial species such as *Bacillus* sp., *Lactobacillus* sp., *Enterococcus* sp., *Carnobacterium* sp. and yeast *Saccharomyces cerevisiae* (Cruz et al 2012). Probiotic microorganisms can originate from the host or from other sources but it is not yet known whether probiotics originating from the host are better than those from other sources (Nguyen et al 2019).

The present research has been carried out from April to September 2019 to evaluate the effects of probiotic isolated from digestive tracks of carp on growth and feed efficiency of such hosts.

Material and Method

Fish. The fish used were carp fingerlings with an average weight of 2.09±0.27 g. The fish was gathered from Freshwater Aquaculture Fisheries Center, Tatelu. The fish taken were pleced in an oxygenated plastic bag, then transported to the Aquaculture Technology Laboratory of the Faculty of Fisheries and Marine Sciences for further use.

Probiotic bacteria. The probiotic used was previously isolated from the intestine of carp weighing about 100 g at the Laboratory of Fish Health, Environment and Toxicology, Faculty of Fisheries and Marine Science. Briefly, one gram of intestine was cut into small pieces, ground using a mortar and added to a 9 mL NaCl. The solution was centrifuged twice and the supernatant was removed. It was then diluted to 10^{-2} and 10^{-3}, spread onto a Petri dish containing MRS agar and placed in an incubator at 28^{o}C for 24-48 hours. The growing bacteria were then purified through several times of reculture in the same media. The bacteria obtained were gram-positive, rod-shaped, and biochemically identified as *Lactobacillus* sp.

Feed preparation. The concentration of probiotics used as treatments consisted of $1x10^{6}$, $1x10^{7}$, $1x10^{8}$ and $1x10^{9}$ cfu mL^{-1}. Each treatment was supplemented by spraying thoroughly to commercial pellets (Hi-Pro-Vite FF999) as much as 1%. The composition of the feed was 35% crude protein, 2% lipid, 3% crude fiber, 13% ash, and 12% water. After air-dried, the feed was coated with 2% yellow eggs, air-dried again, placed into a plastic bag and stored at the refrigerator. Pellet without the addition of probiotics but coated with yellow eggs was used as a control treatment.

Experimental design. The experimental design used in this research was completely randomized design (CRD) with 5 treatments, each with 3 replications. The fish were acclimatized for one week before running the experiment. During the adaptation period, the fish were fed with pellet without the addition of probiotics at a dose of 5%/body weight/day. Feeding frequency was twice a day at 09.00 am and 16.00 pm.

After acclimatization process, the fish were distributed into 15 aquaria (60x40x40 cm each) with a density of 20 individuals per aquarium. Each aquarium was equipped with an aerator and a submersible water pump for recirculation system. Fish were fed probiotic-supplemented pellets that had been previously prepared for 30 days at 5% per

body weight/day, two times a day (09.00 am and 16.00 pm). Uneaten food and wastes of fish were removed by syphoning. Water replacement was also carried out every 2-3 days as much as 30% of the water volume. The parameters measured were weight gain, daily growth rate, feed efficiency, and food conversion ratio. Fish growth and feed efficiency were calculated based on the formula applied by Zokaeifar et al (2012) and Dawood et al (2016) as follows:

$$\text{Weight gain, WG (g)} = \text{Wt} - \text{Wo}$$

Where: Wt: final weight of fish (g)
Wo: initial weight of fish (g)

$$\text{Specific growth rate,} \quad \text{SGR (\%)} = \frac{\ln(\text{Wt} - \text{Wo})}{\text{t}} \; x\, 100$$

Where t: feeding duration (days)

$$\text{Feed efficiency, FE (\%)} = \frac{\text{Wt} - \text{Wo}}{\text{Feed intake (g)}} \; x\, 100$$

$$\text{Feed conversion ratio,} \quad \text{FCR} = \frac{\text{Total feed given (g)}}{\text{WG}}$$

Data analysis. The effect of different concentrations of probiotics on growth and feed efficiency was analyzed through one-way Analysis of variance. The different effect between treatments was analyzed by Duncan's Test using SPSS 24 for windows. The effect was considered significant at $p<0.05$.

Result and Discussion. The addition of probiotics isolated from carp intestine into the diets with different concentrations had a very significant effect on weight gain and specific growth of carp fingerlings ($p<0.01$). After the fish were fed for 30 days, the best weight gain (WG) was achieved in fish fed pellet supplemented with probiotics $1x10^8$ cfu/mL (Treatment D), followed by concentrations of $1x10^7$ cfu/mL (Treatment C). The best specific growth rate (SGR) was also achieved in treatment D, then in treatment C (Table 1). Duncan test showed that WG and SGR of fish treated with treatment D and C were significantly different compared to WG and ADG of control fish (treatment A), as well as with fish treated with treatment E ($1x10^9$ cfu/mL) and B ($1x10^6$ cfu/mL). However, there was no significant difference between treatments D and C. WG and ADG of fish treated with treatment E were significantly different from those of control fish as well as fish in treatment B.

Table 1

Weight gain, specific growth, FCR and FE of fish fed pellet added with probiotic

Parameters	*Treatments*				
	A	*B*	*C*	*D*	*E*
Final weight (g)	3.89±0.39	4.04±0.37	5.64±0.11	6.09±0.10	5.20±0.17
WG (g)	1.80±0.39^a	1.94±0.37^a	3.55±0.11bc	4.00±0.10^c	3.11±0.17^b
SGR (%)	2.20±0.36^a	2.33±0.33^a	3.54±0.07^{bc}	3.82±0.06^c	3.25±0.11^b
FE (%)	48.72±7.57^a	49.55±7.27^a	82.68±1.43^b	89.68±6.44^b	77.73±7.71^b
FCR	2.08±0.32^a	2.05±0.31^a	1.21±0.02^b	1.12±0.08^b	1.29±0.12^b

Means with different superscript in the same row are significantly different. A - control without addition of probiotic; 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}; WG - weight gain (g); SGR - specific growth rate (%); FCR - feed conversion ratio; FE - feed efficiency (%).

The present research proves that probiotics originating from the digestive organs of carp have the potential to spur fish growth. Previous research report showed that probiotic isolated from catfish intestines can increase the growth of carp juveniles (Manoppo et al 2019). Probiotics isolated from the digestive tract of gourami (*Osphronemus goramy*) had

also been reported to increase the growth of tilapia (Mulyasari et al 2016). In red sea bream (*Pagrus major)*, supplementation of *Lactobacillus rhamnosus* in feed and given for 56 days had a significant effect on the increase of fish growth and immunity (Dawood et al 2016). Furthermore, Lin et al (2019) reported that giving *Lactobacillus* spp. to Lined sea horse (*Hippocampus erectus*) had a significant influence on growth, feed utilization, feed digestibility, protease activity, and fish immune response. Several research reports also proved that probiotics derived from fish digestive organs could induce growth, feed conversion ratio and feed efficiency (Mohammadian et al 2019; Doan et al 2018; Marlida et al 2014).

In the fish given probiotic with higher concentration (Treatment E), fish growth tended to be slower than the growth of fish in lower concentration (treatment D and C). This occurred because of the probiotic concentration added had probably exceeded the required dose. Conversely at lower dose (treatment B), administration of probiotic had no effect on the growth. It was suspected that the amount of probiotics given was not enough to function effectively in promoting fish growth. This condition occurred because the administration of an ingredient such as probiotic was strongly influenced by the dose and duration of administration (Sakai 1999).

Supplementation of probiotic into feed with different concentrations also had a very significant effect on feed conversion ratio (FCR) and feed efficiency (FE) ($p<0.01$). After feeding for 30 days, the best FCR value was achieved in fish in treatment D, then C and E. The best FE value was also observed in fish in treatment D, followed by C and E. Based on Duncan's Test, the FCR and FE values in these three treatments were not significantly different one to each other, but significantly different if compared with control fish and fish in treatment B. There were no significant differences between treatments A and B (Table 1).

The present research proved that probiotics isolated from the carp intestine could reduce the FCR and increase the FE value. Similar research reports showed that probiotic (*Lactobacillus* sp.) isolated from digestive organ of catfish and given to carp for 30 days significantly increased FCR and FE (Manoppo et al 2019). Probiotic isolated from the digestive tract of Humpback groupers could also reduce the value of feed conversion, increase growth, protein and fat retention (Marlida et al 2014). In addition, several studies found that the use of probiotics in aquaculture could improve growth, digestibility and feed efficiency, appetite, the fish's immune system, tolerance to stress and resistance to pathogens (Midhun et al 2019; Mulyasari et al 2016; Ling et al 2018; Rahmawan et al 2014; Hemaiswarya et al 2013). The report by Midhun et al (2019) showed the addition of probiotics in feed both single and in combination between *Lactobacillus plantarum* N11 and *Bacillus velezensis* H3.1 improved growth performance, specific growth rate, weight gain, final weight and feed conversion ratio in tilapia. Ullah et al (2018) reported that the use of commercial probiotics significantly increased growth, digestive enzymes activities and immune response of Mori fish (*Cirrhinus mrigala*) in the polyculture system. Furthermore, Tan et al (2019) reported that supplementation of probiotics (*Rummmeliibacillus stabekisii*) isolated from the stomach of tilapia into the feed and given to tilapia (mean 4.1±0.34 g) for 8 weeks increased fish growth, immunity, disease resistance and gut microflora and health status of tilapia. In shrimp, Kongnum & Hongpattarakere (2012) reported that the addition of probiotics isolated from wild shrimp and fed for 6 weeks significantly increased the relative growth rate, reduced FCR and increased the survival of *Penaeus vannamei*, as compared to control shrimp. Wang et al (2019) found that diet supplemented with a mixture of several species of probiotics for 56 days improved growth performance, survival and health status of shrimp (*P. vannamei*). Xie et al (2019) also reported the use of a mixture of probiotics in white shrimp increased growth, FE, nonspecific immune response, digestive enzymes activity, and reduce FCR.

Conclusions. Supplementation of probiotic isolation from digestive tract of carp is able to enhance growth performance and feed efficiency as indicated by the increase in weight gain, specific growth, feed efficiency and reduce food conversion ratio. The best results were obtained in fish fed pellet with the addition of $1x10^8$ cfu mL^{-1}.

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