The use of probiotic isolated from Sangkuriang catfish (*Clarias gariepinus* var. Sangkuriang) intestine to improve growth and feed efficiency of carp, *Cyprinus carpio*

[1]Henky Manoppo, [2]Reiny A. Tumbol, [2]Hengky J. Sinjal, [1]Intan A. Novitarizky

[1] Aquaculture Study Program, Faculty of Fisheries and Marine Science, Sam Ratulangi University, Manado-95115, Indonesia; [2] Aquatic Science Study Program, Graduate School of Sam Ratulangi University, Manado-95115, Indonesia. Corresponding author: H. Manoppo, hmanoppo@yahoo.com

Abstract. The effect of probiotic isolated from sangkuriang catfish (*Clarias gariepinus* var. Sangkuriang) on growth and feed efficiency of carp (*Cyprinus carpio*) has been studied. Carp juveniles weighing 2.13±0.09 g in average were obtained from Freshwater Aquaculture Center Tatelu, North Minahasa Regency. After acclimatization in laboratory condition for two weeks, the juveniles were cultivated in 15 aquaria with a density 15 individuals each. Probiotic used was obtained from the Laboratory of Fish Health, Environment and Toxicology of the Faculty of Fisheries and Marine Science Sam Ratulangi University Manado-Indonesia. Fish were fed with feed added with probiotic bacteria with different concentrations namely $1x10^6$, $1x10^7$, $1x10^8$, $1x10^9$ cfu mL^{-1}. Control food was not supplemented with probiotic. Probiotic-feed was applied for 21 days at 5% of their body weight per day, twice daily. The data collected consisted of average daily growth rate, weight gain, feed efficiency and feed conversion ratio. The results showed that the addition of probiotic bacteria in feed was able to increase the growth, feed efficiency and decrease the feed conversion ratio ($p < 0.01$). The best growth, feed efficiency and feed conversion ratio were achieved in fish fed with the addition of probiotic bacteria $1x10^8$ cfu mL^{-1}. In conclusion, probiotic bacteria isolated from catfish intestine have the potential to improve the growth performance, feed efficiency and decrease feed conversion ratio. The best results were observed in fish fed food supplemented with probiotic suspension containing $1x10^8$ cfu mL^{-1}.
Key Words: probiotic, growth, feed efficiency, aquaculture, immune system.

Introduction. Carp (*Cyprinus carpio*) is one of the freshwater fish species that is very popular for the people of North Sulawesi, Indonesia. High economic value and high demand have encouraged industries to develop the fish farming business from semi-intensive system to more intensive system. Intensification which is not accompanied by adequate technology and management has resulted in stressful condition, deterioration of water quality, poor growth and high prevalence of disease outbreaks (Manoppo et al 2016). The most widely used disease prevention method was using antibiotics and chemotherapy ingredients. The intensively or repeatedly use of antibiotics or chemicals had caused various negative effects such as bioaccumulation, pollution, antibiotic-resistant pathogens, damaging the microbial population of the environment and immunosuppression (Biswas et al 2012; Karthik et al 2014). Antibiotic residues can be accumulated in the fish's body, and are harmful to people health (Payung et al 2017). In Indonesia, the Government of Indonesia since 2015 has banned the use of drugs and chemicals in aquaculture through the Regulation of the Minister of Maritime Affairs and Fisheries concerning Control of Residual Fish Medicines, Chemicals and Contaminants (Pudjiastuti 2015) which are references in monitoring and controlling residues. This is done to improve the quality and competitiveness of aquaculture products.

Thus, scientists are then challenged to provide technologies that increase resistance to disease and growth of aquatic organisms through improvement of feed efficiency in aquaculture industries without damaging the environment (Sahu et al 2008). Researchers have studied and proposed several approaches to accomplish environmental friendly aquaculture. Application of probiotic is one of the most promising technology in preventing infectious disease and the best alternative to the use of chemotherapeutics in aquaculture (Hagi & Hoshino 2009; Das et al 2010). Kesarcodi-Watson et al (2008) stated that the use of probiotic bacteria is beneficial for preventing disease outbreaks, promoting fish growth, improving feed digestibility and feed utilization, and the best alternative to antibiotics and chemicals. Probiotics produce antimicrobial compounds so they can kill or inhibit pathogens (Hagi & Hoshino 2009; Sornplang & Piyadeatsoontorn 2016) and regulate digestive microbial balance (Kolndadacha et al 2013). Probiotics also 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 (Kurniasih et al 2013; Lara-Flores & Olvera-Novoa 2013). 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 pathogen (Irianto & Austin 2002; Aly et al 2008; Gobinath & Ramanibai 2012; Hemaiswarya et al 2013; Rahmawan et al 2014; Mulyasari et al 2016; Zhang et al 2018).

Probiotics are living microorganisms that when administered to animals, humans or fish will affect the host by improving the indigenous micro flora properties of the host or the environment (Kolndadacha et al 2013). FAO (2002) defines probiotics as living microorganisms which when given in the right amount can improve the health of the host. According to Verschuere et al (2000), probiotics is a living microbial agent that gives a beneficial effect on the host, therefore probiotic bacteria should not be pathogenic to the host under normal circumstances or stress. In aquaculture, probiotic is an essential tool for fish health management (Akter et al 2016). Probiotics are also used as therapeutic, prophylactic and supplement growth in animal production and human health (Kesarcodi-Watson et al 2008). Generally probiotics are included in the genera of *Lactobacillus, Bacillus, Bifidobacterium, Vibrio, Saccharomyces, Enterococcus, Bacillus subtilis* (Pannu et al 2014). Research results had confirmed that application of probiotic in aquaculture was potential to enhance health status of fish through modulating the immune system and thus increase resistance to disease and improve environment quality through balancing micro flora in the water (Fuller 1989; Verschuere et al 2000). Iribarren et al (2012) stated that the use of probiotic bacteria is one of the internal solutions to reduce environmental damage due to the accumulation of wastes in aquaculture media, produce optimal growth and feed efficiency, and reduce production costs. Thus application of probiotic is urgently required for environmental friendly aquaculture. This research was conducted to study the potential of probiotic isolated from sangkuriang catfish to improve growth performance and feed efficiency of carp.

Material and Method

Experimental fish*.* The fish used were carp (*Cyprinus carpio*) juveniles measuring 3-5 cm with an average weight of 2.13±0.09 g. Fish were obtained from Freshwater Aquaculture Center at Tatelu Village, put in plastic bags and transported to Laboratory of Aquaculture Technology at Faculty of Fisheries and Marine Science, Sam Ratulangi University. The research was conducted from May to September 2018.

Probiotic bacteria*.* Probiotic bacteria (*Lactobacillus* sp.) were obtained from Laboratory of Fish Health, Environment and Toxicology at The Faculty of Fisheries and Marine Science, University of Sam Ratulangi. The probiotic was isolated previously from the intestine of sangkuriang catfish (*Clarias gariepinus* var*.* Sangkuriang).

Feed preparation*.* The probiotic was cultured on MRS medium for 48 hours at 28°C. The probiotic colonies were suspended in NaCl 85% to get the concentration of $1x10^9$, $1x10^8$, $1x10^7$ and $1x10^6$ cfu mL^{-1}. Each solution was added as much as 1% to commercial feed (Hi-Pro-Vite FF999 by CV. Prima) containing crude protein 35%, lipid 2%, crude fiber 3%, ash 13%, and water 12%. The mixture was air-dried, coated with egg white, air-dried again and stored at refrigerator until use. Control food was not supplemented with probiotic solution but coated only with egg white.

Research procedure and data collection. The study used a completely randomized design (CRD) with 5 treatments namely treatment A = control diet; B = diet supplemented with 1% probiotic suspension $1x10^6$ cfu mL^{-1}; C = diet supplemented with 1% probiotic suspension $1x10^7$ cfu mL^{-1}; D = diet supplemented with 1% probiotic suspension $1x10^8$ cfu mL^{-1}; E = diet supplemented with 1% probiotic suspension $1x10^6$ cfu mL^{-1}, each with 3 replications. Before treated, the fish were first adapted for one week in 15 aquaria (60 x 40 x 40 cm) with a density of 25 individuals $aquarium^{-1}$. Each aquarium was supplied with an aerator and recirculation system using small submersible water pump. Uneaten food and fish feces were removed once a day by syphoning. Water replacement was also carried out every 2-3 days as much as 30% of the water volume.

After acclimatization, the density of fish in each aquarium was set to 15 individuals. Fish were fed with feed mixed with probiotics already prepared as treatments for 21 days with a frequency of 2 times a day (08.00 am and 16.00 pm) as much as 5% of body weight per body day. Parameters measured were weight gain, average daily growth (ADG), feed efficiency (FE), and food conversion ratio (FCR). All the parameters were calculated based on the following formula (Marlida et al 2014; Mulyasari et al 2016) as follows:

$$\text{Weight gain (g)} = \text{Final weight (g)} - \text{Initial weight (g)}$$

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

$$\text{Feed efficiency,} \quad \text{FE (\%)} = \frac{\text{Final weight (g) - Initial weight (g)}}{\text{Feed intake (g)}} \times 100$$

$$\text{Feed conversion ratio,} \quad \text{FCR} = \frac{\text{Total feed intake (g)}}{\text{Weight gain (g)}}$$

Data analysis. Analysis of variance was applied to evaluate the effect of different probiotic concentration on weight gain, ADG, FE, and FCR while the different effect between concentrations was analyzed by Duncan's Test using SPSS 24 for Windows. Comparisons were made at $p < 0.05$.

Results and Discussion

Weight gain. The addition of probiotic bacteria with different concentrations into the diet displayed a significant effect on weight gain of fish ($p < 0.01$). After 21 days of feeding, the highest weight gain was observed in treatment D where the fish fed diet supplemented with probiotic suspension $1x10^8$ cfu mL^{-1} (Table 1). In this treatment, weight gain of fish was about 43.45% greater than weight gain in control fish (treatment A). Statistically, weight gain in treatment D was significantly different as compared to other treatments except with treatment E where there was no differences between the two.

Table 1

Growth performance and feed efficiency of carp fed probiotic-diets

Treatments	*Final weight (g)*	*Weight gain (g)*	*ADG (%)*	*FE (%)*	*FCR*
A	4.04	1.91 ± 0.245^{a}	3.04 ± 0.2^{a}	52.627 ± 5.86^{a}	0.9 ± 0.03^{c}
B	4.29	2.16 ± 0.133^{ab}	3.34 ± 0.1^{ab}	59.49 ± 2.06^{a}	0.84 ± 0.02^{bc}
C	4.30	2.16 ± 0.191^{ab}	3.33 ± 0.2^{ab}	57.88 ± 4.19^{a}	0.87 ± 0.02^{c}
D	4.87	2.74 ± 0.245^{c}	3.93 ± 0.2^{c}	72.86 ± 6.34^{b}	0.77 ± 0.05^{a}
E	4.70	2.57 ± 0.27^{bc}	3.76 ± 0.3^{bc}	69.06 ± 2.82^{b}	0.79 ± 0.01^{ab}

Means having different superscript in the same column are significantly different; ADG = average daily growth; FE = feed efficiency; FCR = food conversion ratio; treatment A = control diet; B = diet supplemented with 1% probiotic suspension $1x10^{6}$ cfu mL^{-1}; C = diet supplemented with 1% probiotic suspension $1x10^{7}$ cfu mL^{-1}; D = diet supplemented with 1% probiotic suspension $1x10^{8}$ cfu mL^{-1}; E = diet supplemented with 1% probiotic suspension $1x10^{6}$ cfu mL^{-1}.

Average daily growth. As with the weight gain, the addition of probiotic in feed had significant effect on daily growth of fish ($p < 0.01$). The best daily growth was achieved in fish fed diet added with probiotic suspension in treatment D, followed by treatment E. Even though the daily growth of fish in treatments D and E was not different statistically, but the value in treatment E tended to decrease, indicating an over doses (Table 1). On the other hand, at lower concentrations, the probiotic bacteria probably had not been able to function effectively resulting in a lower growth. Sakai (1999) stated that administration of an ingredient such as probiotic is influenced by dose and duration time of administration. Excessive doses might not be able to promote growth but instead suppress the growth.

Feed efficiency and feed conversion ratio. Research results found that probiotic-diet fed for 21 days had significant effect on FE and FCR. FE of fish in treatment D was higher and significantly different as compared to that of control fish ($p < 0.01$). However, there was no significant different observed between treatment D and E (Table 1). The lowest FCR was achieved in treatment D. Thus, probiotic isolated from sangkuriang catfish used in this research was potential to improve growth and feed efficiency.

Several researches had also reported that probiotics originating from gastrointestinal tract of fish and shrimp improved growth. A research by Mulyasari et al (2016) reported that probiotic originating from the gastrointestinal tract of gouramy (*Osphronemus goramy*) had the ability to increase the growth of Nile tilapia (*Oreochromis niloticus*). The results showed that tilapia fingerling fed feed added with probiotic had better growth than control fish. Marlida et al (2014) also reported that probiotic isolated from humpback groupers (*Cromileptes altivelis*) digestive organ induced growth, protein and fat retention and reduced FCR. In shrimp, Widanarni et al (2015) reported that probiotics isolated from gastrointestinal tract of Pacific white shrimp (*Litopenaeus vannamei*) could promote growth, protein digestibility and protein storage of shrimp. Probiotics will enter the digestive system of the fish and then attach to the intestine and use a large amount of carbohydrates for the growth. The bacteria also produce digestive enzymes that increase the digestibility of organic materials and proteins. Probiotic *Lactobacillus* sp. was also reported to reduce mortality of *P. monodon* and *L. vannamei* against vibriosis (Karthik et al 2014).

Lara-Flores & Olvera-Novoa (2013) proved that the increase in growth performance of fish occurred due to the balance of micro flora in the gastrointestinal tract that leads to an increase quality of feed absorption and digestive enzymes such as amylase, protease, lipase and cellulose enzymes. Similarly, Hemaiswarya et al (2013) reported that probiotics could improve fish growth through stimulating fish appetite and increasing nutritional value by producing vitamins, detoxifying compounds in the diet and simplifying the compounds so it will be easily digested by fish. The results of Parameswari et al (2013) showed that fish (*Channa striata*) kept in the media with the addition of probiotic had higher survival and better growth than control fish.

In a study conducted by Gobinath & Ramanibai (2012), the application of probiotic *Lactobacillus* sp. isolated from *Oreochromis mossambicus* in feed significantly increased the size and weight of infected tilapia as compared with control fish that fed feed without addition of probiotic. The research by Rahmawan et al (2014) revealed that the addition of probiotic in feed increased feed utilization, growth and survival of Dumbo catfish (*Clarias gariepinus*). Doan et al (2018) found that the use of diet supplemented with singular or combined *Lactobacillus plantarum* N11 and *Bacillus velezensis* H3.1 improved the growth performance, specific growth rate, weight gain, final weight, and feed conversion ratio ($p < 0.05$) of Nile tilapia (*O. niloticus*).

Conclusions. This research found that the inclusion of probiotic bacteria isolated from sangkuriang catfish into feed was able to improve growth and feed efficiency of carp. The best results were observed in fish fed food supplemented with probiotic suspension containing $1x10^8$ cfu mL^{-1}.

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Authors:
Henky Manoppo, Aquaculture Study Program, Faculty of Fisheries and Marine Science, Sam Ratulangi University, Manado-95115, Indonesia, e-mail: hmanoppo@yahoo.com
Reiny A. Tumbol, Aquatic Science Study Program, Graduate School of Sam Ratulangi University, Manado-95115, Indonesia, e-mail: reinytumbol@yahoo.com
Henky J. Sinjal, Aquatic Science Study Program, Graduate School of Sam Ratulangi University, Manado-95115, Indonesia, e-mail: juliushengky@yahoo.com
Intan A. Novitarizky, Aquaculture Study Program, Faculty of Fisheries and Marine Science, Sam Ratulangi University, Manado-95115, Indonesia, e-mail: intan.alam16@gmail.com

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