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Effect of the administration of probiotic *Bacillus* NP5 in the rearing media on water quality, growth, and disease resistance of African catfish (*Clarias gariepinus*)

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Abstract. Putra AN, Syamsunarno MB, Ningrum W, Jумыnah, Mustahal. 2020. Effect of the administration of probiotic *Bacillus* NP5 in the rearing media on water quality, growth, and disease resistance of African catfish (*Clarias gariepinus*). *Biodiversitas* 21: 2566-2575. The purpose of this study was to investigate the effect of administration of the probiotic *Bacillus* NP5 in rearing media on water quality, growth, and disease resistance of African catfish. The study consisted of three different concentrations of probiotic *Bacillus* NP5 - C: control, A: probiotic *Bacillus* NP5 of 1×10^9 CFU/mL, and B: probiotic *Bacillus* NP5 of 1×10^{10} CFU/mL, on the rearing media of African catfish for 45 days. Based on the results, the lowest significant ammonia ($P < 0.05$) was found in treatment A (1.91 ± 0.17 mg/L), then followed by B (2.12 ± 0.14 mg/L) while the highest was in the control (2.36 ± 0.19 mg/L). Treatment A also had a better immune response compared to other treatments shown with the highest significant number of leukocytes at $6.69 \pm 0.17 \times 10^4$ cells/mm³ and phagocyte activity at $65.19 \pm 0.04\%$. Furthermore, treatment A significantly resulted in the best specific growth rate and feed conversion ratio ($2.02 \pm 0.07\% \cdot \text{day}^{-1}$, 1.05 ± 0.07 , respectively). The administration of probiotic *Bacillus* NP5 of 1×10^9 CFU/mL in rearing media significantly improved the ammonia value, growth, and resistance of African catfish to *Aeromonas hydrophila* infection.

Keywords: *Bacillus* NP5, *Clarias gariepinus*, disease-resistant, probiotic, water quality

INTRODUCTION

Clarias gariepinus, commonly known as African catfish, is a species of fish widely cultivated in Indonesia (Ngaddi et al. 2019; Iswanto et al. 2019; Nasrulloh et al. 2019) with the production of over 19 thousand tons in 2017 (Ministry of Marine Affairs and Fisheries of the Republic of Indonesia 2019). Based on a report released by FAO in 2018, various intensive fish culture systems have been carried out which help in meeting up for the increasing demands for fish. In intensive culture systems, fish are easily stressed because fish are reared in high density (Cerezuela et al. 2012; Giri et al. 2014) and many accumulations of organic matter originating from leftover feed and feces (Ren et al. 2019). Also, a study conducted by Widanarni et al. (2010) revealed that the accumulation of organic waste generally in this system with high fish density and feed inputs. Similarly, Avnimelech and Ritvo (2003) reported that 75-80% of the feed is excreted into the water in the form of ammonia, considering the fact that fish only assimilate 20-25% in their body. The presence of ammonia in water further increase fish's susceptibility to bacterial infection and could lead to mass death at concentration > 0.2 mg/L (Boyd and Mcnevin 2014). Also, research by Wang et al. (2008), showed that lack of quality water is a major cause of mass death in intensive fish culture. Zhang et al. (2016) and Garcia-Mendoza et al.

(2019) also reported that the health status of fish and shrimp depends on water quality. Poor water quality directly influences fish metabolism, such as decreased growth, stress, increasing the general mortality, and also thereby leading to the spread of disease outbreaks (Xiong et al. 2016). In addition, disease control in intensive aquaculture is a current issue that has ultimately affect the economy of the country (Nandi et al. 2017). Hence, creating a friendly environment in terms of providing quality water to prevent disease attacks (Ramadhani et al. 2019; Amin et al. 2019).

According to Gomez-Gil et al. (2000) and Kuebutornye et al. (2019), the probiotic is an environmentally friendly method with improved water quality, thereby increasing fish immunity against pathogenic attacks. In clear terms, probiotic is an additional living microbe which could benefit its host by improving microbial balance in the digestive tract (Makled et al. 2019; Zhao et al. 2019; Darafsh et al. 2019; Arani et al. 2019; Valipour et al. 2019), increasing feed use or nutritional value, as well as the host response to disease through improved water quality (Verschure et al. 2000). Also, Devaraja et al. (2013) reported that the provision of probiotics helped in improving the quality of water in vannamei shrimp ponds through the mechanism of bioremediation. Based on research conducted by Dawood et al. (2018), bioremediation is the process of decomposing harmful

69) anic wastes in waters using microorganisms. The application of probiotics in various aquaculture systems has been reported to increase water quality through bio-24) rmediation of organic waste in water, thereby reducing the grow-22) of pathogenic bacteria (Nimrat et al. 2012; Devaraja et al. 2013; Zorriehzahra et al. 2016; Chumpol et al. 2017; Elsabagh et al. 2018). The methods of providing probiotic in either rear-15) media (Kewcharoen and Srisapoomee 2019; Zhang et al. 2019; Xie et al. 2019) or feed (Jang et al. 2019; Yang et al. 2019; Tachibana et al. 2019; Tsai et al. 2019) have been found to minimize the occurrence of pathogen attacks and water quality degradation.

Similarly, the results of previous studies showed that applying probiotic *Bacillus NP5* could increase the growth and activity of proteolytic, lipolytic, and amylolytic enzymes of African catfish (Putra and Romdhonah 2019). 2) According to Putra and Widanarni (2015), the probiotic *Bacillus NP5* is usually isolated from the digestive tract of tilapia which has passed the stages of the probiotic selection pro-26) . The research conducted by Tamamdsturi et al. (2016) found that the addition of probiotic *Bacillus NP5* in the feed could increase the growth and immune response of *Pangasianodon hypophthalmus* catfish against *A. hydrophila* infec-14) . Other positive applications of this bacteria in the diet have been found to improve growth performance in tilapia fish (Utami et al. 2015; Putra et al. 2015; Agung et al. 2015), 3) namei shrimp (Widanarni et al. 2014) and improve the immune response of the shrimp against white spot syndrome virus (WSSV) attacks (F-9) rianti et al. 2016). However, no research has shown the role of probiotic *Bacillus NP5* in the rearing media of fish culture. Therefore, this study aimed to investigate the eff-76) of the administration of the probiotic *Bacillus NP5* on water quality, growth, and disease resistance of African catfish in the rearing media.

MATERIALS AND METHODS

Culture of probiotic *Bacillus NP5*

Isolates of the probiotic *Bacillus NP5* were obtained from the Laboratory of Fish Health, Bogor Agricultural University. A total of 250 mL of TSB (Tryptic soy broth) media was prepared and isolate of *Bacillus NP5* bacterium was inoculated on it and incubated for 18 hours at 29°C (Putra and Romdhonah 2019). Next, the freshly harvested culture was centrifuged at a speed of 1000 g for 10 minutes. Then probiotics were diluted serially by adding Phosphate Buffered Saline (PBS) based on different treatments (Nurhayati et al. 2015), after which these diluted samples were given to the catfish through the rearing media.

Experimental design

10) This study was conducted at Wet Laboratory, Department of Fisheries, Faculty of Agriculture, University of Sultan Ageng Tirtayasa. The African catfish used were the seeds from the Baros Fish Seed Center (BBI Baros),

30) Serang Regency, Banten. Each fish with an average w-34) t of 8.06 ± 0.06 g were reared for 45 days using a round tank with a diameter of 71 cm and a height of 53.5 cm involving 20 fish/tank. The fish acclimation was carried out for 5 days be-55) - to the rearing process. Experimental fish were 33) d the commercial feed with a protein content of 30% and fed to fish three times a day (08:00, 12:00, 16:00 Western Indonesia Time) up to satiation. In addition, the water purification and replacement processes were not-19) ried out during rearing. The study design was the completely randomized design consisting of three treatments with different concentrations of the probiotics on the rearing media and three replications, namely:

C: control

A: probiotic *Bacillus NP5* of 1×10^9 CFU/mL

B: probiotic *Bacillus NP5* of 1×10^{10} CFU/mL

Th-36) challenge test

The pathogenic bacteria used, *A. hydrophila*, was obtained from the Laboratory of Fish Health, IPB University. Then, Koch's postulate was carried out to increase its virulence. Furthermore, *A. hydrophila* was 42) culated in 50 mL TSB media and then incubated at 20 °C for 24 hours. The bacterial culture was subsequent-50) ly centrifuged at 1000 g for 10 minutes and then diluted to a density of 10^7 CFU/mL through the addition of PBS. The pathogenic bacterium was then injected into catfish intramuscularly (Putra and Widanarni 2015) at a dose of 10^7 CFU/mL. The wound formed due to this was scratched on the TSA media using the quadrant scratch method.

Subsequently, Lethal Doses 50% (LD₅₀) was performed to obtain the amount of the bacteria used in the challenge test. LD₅₀ is the pathogen needed to kill 50% of the total catfish population. In addition, the pathogenic bacterium was 42) culated on 100 mL TSB media, then incubated at 20 °C for 24 hours. Further, the bacterial culture was centrifuged at 1000 g for 10 minutes to obtain fresh samples and then 37) en the catfish rearing media with 3 different doses - 10^5 CFU/mL, 10^6 CFU/mL, and 10^7 CFU/mL. After 10 days of observation, the test results showed that the treatment with a dose of 10^6 CFU/mL had a survival rate of 55%. Hen-57) ce the sample with a dose of 10^6 CFU/mL was used in the challenge test.

The challenge test was carried out after 30 days of rearing period with the addition of probiotic *Bacillus NP5* in the rearing media. The *A. hydrophila* isolate was inoculated on 200 mL TSB media and then cultured on a water bath shake-2) at 29°C for 24 hours. Furthermore, the bacterial culture was centrifuged at a speed of 1000 g for 10 minutes after which the fresh culture was further diluted using PBS to a density of 10^6 CFU/mL and given to catfish rearing media.

Water quality parameters

The water quality sampling was carried out on days 0, 10, 20, 30, 34, 38 and 45 which involved the measurements of temperature and Dissolved Oxygen (DO) with the use of a DO meter with a brand of Luxtron DO550, and pH using a pH meter with a type of lux-10) 208, in-situ manner. The ammonia measurements were carried out at the Laboratory

of Aquaculture, Department of Fisheries, Faculty of Agriculture, University of Sultan Ageng Tirtayasa. A sample of 100 mL of water was taken from each container and measured using a spectrophotometer AMV01 through the Phenate standard method following with APHA (1988).

Growth parameters

The growth sampling was carried out on Day 30 before the challenge test and at the end of the culture or after the challenge test (Day 45). The fish were weighed and counted at the beginning and end of the rearing process to calculate the value of the Specific Growth Rate (SGR) and Survival Rate (SR). The amount of the initial and final feeds were weighed to obtain the value of total feed consumption (FI) and Feed Conversion Ratio (FCR). Calculations of SGR, SR, FI, and FCR were in accordance with Huisman (1987) as follows:

$$\text{SGR (\% day}^{-1}\text{)} = ((\text{Ln Bt} - \text{Ln Bo})/\text{P}) \times 100$$

$$\text{SR (\%)} = (\text{Et}/\text{Eo}) \times 100$$

$$\text{FI (g)} = \text{Ft} - \text{Fo}$$

$$\text{FCR} = \text{FI}/(\text{Bt} + \text{Bd} - \text{Bo})$$

Note: Bt: t-th biomass of fish, Bo: initial biomass of fish, Et: t-th number of fish, P: time (days), Eo: initial number of fish, Ft: t-th amount of feed, Fo: the initial amount of feed, Bd: biomass of dead fish.

Hematological parameters

A total of 3 fish from each treatment was selected and blood samples were taken on days 0, 30 (before the challenge test) and 45 (after the challenge test), with a syringe already containing anti-coagulant, 0.1 mL of 3.8% Na citrate. Furthermore, blood profile consisting of hematocrit and hemoglobin levels, total erythrocytes and leukocytes, and phagocytic activity were calculated with the aid of a microscope. Determination of hematocrit levels and phagocytic activity were in accordance with Anderson and Siwicki (1995) method, hemoglobin levels were measured through the Sahli method in Wedemeyer and Yasutake (1977), and the total erythrocytes and leukocytes were following Blaxhall and Daisley (1973) method.

Histopathology

Histology was carried out to evaluate the level of organ damage due to pathogen attacks based on the method described by Wada et al. (2011). Its preparation was made at the Histopathology Laboratory, Fish and Environmental Disease Examination Station (LP2IL), Serang, Banten Province. At the end of the culturing period, 3 catfish were taken from each treatment for histological observation. This was carried out on the gills, kidneys, and liver, then fixed using a 10% phosphate-buffered formalin solution and 5% ethylenediaminetetraacetic acid (EDTA). The histology preparation was then stained with hematoxylin and eosin (H&E) and Gram stains.

Data analysis

The growth data and hematological parameters obtained at the end of rearing were analyzed using Analysis of Variance (ANOVA) and for those with differences were subjected to Duncan multiple range tests with a 95%

confidence interval (Duncan 1995). In addition, the results of histological observations were analyzed descriptively.

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RESULTS AND DISCUSSION

Water quality

The variation of water quality in African catfish rearing is shown in Table 1. It shows that there was no significant difference ($P > 0.05$) of ammonia value in catfish rearing media before (H0, H10, H20, and H30) and after challenge tests (H34, H38). The difference in ammoniac value occurred on day 45, and the lowest significant ammonia value ($P < 0.05$) was in treatment A, which was 1.91 ± 0.17 mg/L, then treatment B was 2.12 ± 0.14 mg/L and the highest ammonia value was found in the control treatment, which was 2.36 ± 0.19 M. Furthermore, the addition of probiotics in African catfish rearing had no effect ($P < 0.05$) on the parameters of temperature, DO and pH. The values of the temperature range in the catfish rearing from day 0 to 45 ranged between 28.0 and 29.0 °C and the DO ranged between 5.1 and 6.9 mg/L, while the range of the pH was 6.7-7.0.

Growth

The effect of the addition of the three different dosages of the probiotic *Bacillus* NP5 on the growth performance of African catfish is presented in Table 2. The results showed that the highest significant African catfish biomass on the 30th day ($P < 0.05$) was found in treatments A and B (46.00 ± 7.30 g, and 334.17 ± 12.28 g, respectively), while the lowest biomass was in the control treatment at 251.43 ± 10.73 g. The amount of feed consumption and survival rate of African catfish on the 30th day also did not differ ($P > 0.05$) among the treatments. The highest significant SGR and FCR values on the 30th day ($P < 0.05$) were found in the treatments with the addition of probiotics compared with the control. The highest significant FCR value ($P < 0.05$) on the 30th day was also found in the probiotic treatments compared with the control. In a period of 30-45 days or after the challenge test, the highest final significant biomass value of catfish ($P < 0.05$) was found in treatment A (398.97 ± 12.12 g), then followed by B (369.57 ± 11.23 g) while the lowest was found in the control treatment (270.37 ± 5.90 g). The highest significant FCR value ($P < 0.05$) was found in the control treatment compared with those with probiotic. The results of the survival rates were found, where the highest was found in the treatments with probiotics (A = $89.82 \pm 0.30\%$ and B = $83.07 \pm 7.56\%$) compared with the SR from the control, which was $50.96 \pm 6.50\%$. In the overall time period, day 0 to 45, the highest significant biomass and SGR value ($P < 0.05$) was found in treatment A, then followed by B before the control. The SGR value obtained for treatment A was $2.02 \pm 0.07\%$ day⁻¹, while the values for control and treatment B were $1.15 \pm 0.05\%$ day⁻¹ and $1.85 \pm 0.07\%$ day⁻¹, respectively. However, the highest significant FCR value ($P < 0.05$) was found in the control treatment, while the lowest found in treatment A. Furthermore, the addition of probiotics in the rearing media had a significant effect (P

<0.05) on the survival rate of African catfish. Based on the results, the highest SR value was obtained in the treatments with probiotics supplementation.

Hematological parameters

The results showing the hematological parameters such as the values of erythrocytes, leukocytes, hemoglobin, hematocrit, and phagocyte activity are shown in Table 3. Based on Table 3, the values did not differ among the treatments on day 0. However, there were highest significant values (P <0.05) of erythrocytes, hematocrit and phagocyte activity on day 30 found in the treatment with the probiotic supplementation, and it was more in A compared with B. Then on day 45, after the challenge test, the highest significant values of erythrocytes, leukocytes and phagocyte activity (P <0.05) were found in treatments A and B. However, the highest significant value (P <0.05)

of the leukocyte was found in treatment A at $6.69 \pm 0.17 \times 10^4$ cells/mm³ compared with B at $6.46 \pm 0.06 \times 10^4$ cells/mm³ and control at $5.08 \pm 0.07 \times 10^4$ cells/mm³. The same trend occurred for phagocyte activity where the highest significant value (P <0.05) was found in treatment A at $65.19 \pm 0.04\%$ compared with the control and treatment B at $32.21 \pm 0.18\%$ and $44.20 \pm 0.17\%$, respectively.

Histopathology

Histopathology is a microscopic search of disease in an organism, however, a histopathological observation involves obtaining information in the form of an organ/tissue change pictures. The histopathology results after the challenge test on the gills, liver, and kidneys of catfish are presented in Figures 1, 2, and 3.

Table 1. Water quality values of African catfish (*C. gariepinus*) cultured with the administration of different probiotics in the rearing media

Parameter	Treatment*			Range	Quality standards
	Control	A	B		
Ammonia (mg/L)					
H0	0.01±0.01	0.01±0.02	0.01±0.01	0.01-0.02	<2.5 mg/L**
H10	0.29±0.44	0.04±0.04	0.05±0.08	0.01-0.79	
H20	0.68±0.09	0.65±0.12	0.66±0.11	0.24-0.75	
H30	1.65±0.23	1.34±0.28	1.30±0.21	1.20-1.91	
H34	1.85±0.09	1.52±0.19	1.60±0.23	1.35-1.95	
H38	2.05±0.17	1.75±0.32	1.92±0.10	1.41-2.17	
H45	2.36±0.19 ^b	1.91±0.17 ^a	2.12±0.14 ^{ab}	1.75-2.44	
Temperature (°C)					
H0	28.00±0.00	28.00±0.00	28.00±0.00	28.0-28.0	26-32 °C**
H10	28.77±0.29	28.77±0.12	28.80±0.10	28.0-28.9	
H20	28.43±0.21	28.83±0.06	28.87±0.06	28.2-28.9	
H30	28.53±0.35	28.93±0.06	28.87±0.06	28.2-29.0	
H34	28.80±0.10	28.77±0.21	28.83±0.21	28.6-29.0	
H38	28.93±0.06	28.93±0.06	29.00±0.00	28.9-29.0	
H45	29.00±0.00	28.70±0.26	28.87±0.15	28.5-29.0	
DO (mg/L)					
H0	6.40±0.01	6.41±0.01	6.40±0.01	6.40-6.41	> 3 mg/L**
H10	6.43±0.38	6.57±0.49	6.83±0.21	6.00-6.90	
H20	6.20±0.26	6.03±0.06	6.07±0.06	6.00-6.50	
H30	6.03±0.06	6.17±0.16	6.00±0.00	6.00-6.50	
H34	6.20±0.17	6.13±0.21	6.05±0.35	6.10-6.50	
H38	6.17±0.25	6.23±0.29	6.27±0.32	6.00-6.60	
H45	5.23±0.35	5.27±0.36	5.26±0.12	5.10-6.10	
pH					
H0	7.00±0.00	7.00±0.00	7.00±0.00	7.00-7.00	6-9**
H10	7.00±0.00	7.00±0.00	7.00±0.00	7.00-7.00	
H20	6.90±0.00	6.90±0.00	6.90±0.00	6.90-6.90	
H30	6.90±0.00	6.80±0.00	6.90±0.00	6.80-6.90	
H34	6.90±0.00	6.90±0.00	6.90±0.00	6.90-6.90	
H38	6.74±0.00	6.73±0.06	6.70±0.00	6.70-6.80	
H45	6.80±0.00	6.80±0.00	6.70±0.00	6.70-6.80	

Note: * The same superscript letter in the same row shows the effect of probiotic *Bacillus NP5* that are not different (P>0.05) on the water quality values of catfish rearing. ** Bhatnagar A & Devi P. 2013

Table 2. Growth performance values of African catfish (*C. gariepinus*) cultured with the administration of different probiotics in the rearing media

Day/parameter**	Treatment*		
	Control	A	B
0-30 days (before the challenge test)			
Initial biomass (g)	160.80±0.40	160.53±0.23	160.73±0.31
Final biomass (g)	251.43±10.73 ^a	346.00±7.30 ^b	334.17±12.28 ^b
Feed intake (g)	193.30±14.39	185.87±5.13	188.03±6.93
SGR (%.day ⁻¹)	1.49±0.14 ^a	2.56±0.08 ^b	2.44±0.13 ^b
FCR	2.14±0.13 ^b	1.00±0.14 ^a	1.09±0.07 ^a
SR (%)	98.33±2.89	98.33±2.89	98.33±2.89
30-45 days (after the challenge test)			
Initial biomass (g)	251.43±10.73 ^a	346.00±7.30 ^b	334.17±12.28 ^b
Final biomass (g)	270.37±5.90 ^a	398.97±12.12 ^c	369.57±11.23 ^b
Feed intake (g)	65.57±2.29	63.07±2.36	62.47±2.67
SGR (%.day ⁻¹)	0.49±0.14 ^a	0.95±0.15 ^{ab}	0.67±0.16 ^{ab}
FCR	3.61±0.84 ^b	1.22±0.23 ^a	1.84±0.53 ^a
SR (%)	50.96±6.50 ^a	89.82±0.30 ^b	83.07±7.56 ^b
0-45 days			
Initial biomass (g)	160.80±0.40	160.53±0.23	160.73±0.31
Final biomass (g)	270.37±5.90 ^a	398.97±12.12 ^c	369.57±11.23 ^b
Feed intake (g)	258.87±16.11	248.93±6.90	250.50±6.25
SGR (%.day ⁻¹)	1.15±0.05 ^a	2.02±0.07 ^c	1.85±0.07 ^b
FCR	2.36±0.07 ^c	1.05±0.07 ^a	1.20±0.04 ^b
SR (%)	50.00±5.00 ^a	88.33±2.89 ^b	81.67±7.64 ^b

Note: *The same superscript letter in the same row shows the effect of probiotic *Bacillus* NP5 that are not different ($P > 0.05$) on the growth of catfish. ** SGR (Specific Growth Rate), FCR (Feed Conversion Ratio), SR (Survival Rate)

Table 3. The value of blood profiles in of African catfish (*C. gariepinus*) cultured with the administration of different probiotics in the rearing media

Day/parameter	Treatment*		
	Control	A	B
0 day			
Erythrocytes (x 10 ⁶ cell/mm ³)	2.81±0.05	2.72±0.14	2.81±0.02
Leukocytes (x 10 ⁴ cell/mm ³)	1.28±0.08	1.42±0.04	1.26±0.14
Hemoglobin (g%)	5.79±0.28	6.09±0.51	5.87±0.53
Hematocrit (%)	16.19±0.03	15.79±0.74	16.10±0.15
Phagocyte activity (%)	28.31±0.06	28.44±0.05	28.30±0.07
30 days (before the challenge test)			
Erythrocytes (x 10 ⁶ cell/mm ³)	1.31±0.03 ^a	3.62±0.08 ^c	1.57±0.04 ^b
Leukocytes (x 10 ⁴ cell/mm ³)	5.05±0.04 ^b	7.14±0.10 ^c	4.44±0.29 ^a
Hemoglobin (g%)	5.86±0.31 ^a	7.03±0.66 ^b	5.57±0.16 ^a
Hematocrit (%)	14.77±0.21 ^b	15.93±0.36 ^c	11.82±0.21 ^b
Phagocyte activity (%)	35.42±0.03 ^a	93.19±0.06 ^c	55.28±0.08 ^b
45 days (after the challenge test)			
Erythrocytes (x 10 ⁶ cell/mm ³)	1.42±0.04 ^a	2.67±0.10 ^c	2.48±0.08 ^b
Leukocytes (x 10 ⁴ cell/mm ³)	5.08±0.07 ^a	6.69±0.17 ^c	6.46±0.06 ^b
Hemoglobin (g%)	5.58±0.39 ^a	7.26±0.99 ^{ab}	6.03±0.49 ^b
Hematocrit (%)	8.48±0.33	8.20±0.23	8.14±0.13
Phagocyte activity (%)	32.21±0.18 ^a	65.19±0.04 ^c	44.20±0.17 ^b

Note: *The same superscript letter in the same row shows the effect of the probiotic *Bacillus* NP5 that are not different ($P > 0.05$) on the blood profiles of catfish.

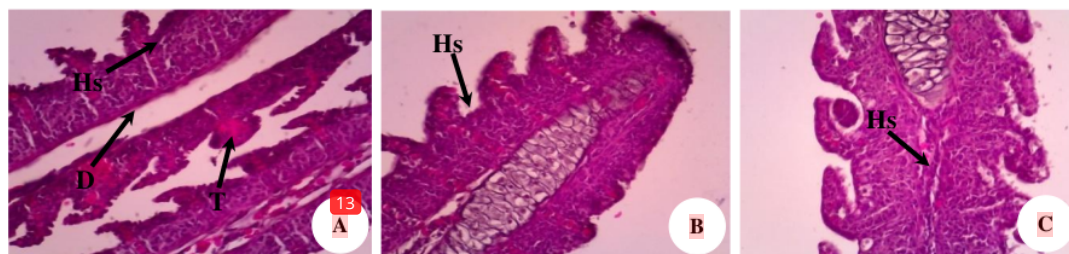


Figure 1. Histopathology of the gill of African catfish (*C. gariepinus*). (Magnification of 400x). The control (C) treatment shows the condition of histological changes in the form of hyperplasia (Hs), desquamation (D), and telangiectasia (T). Treatment A and B show the condition of histological changes in the form of hyperplasia (Hs)

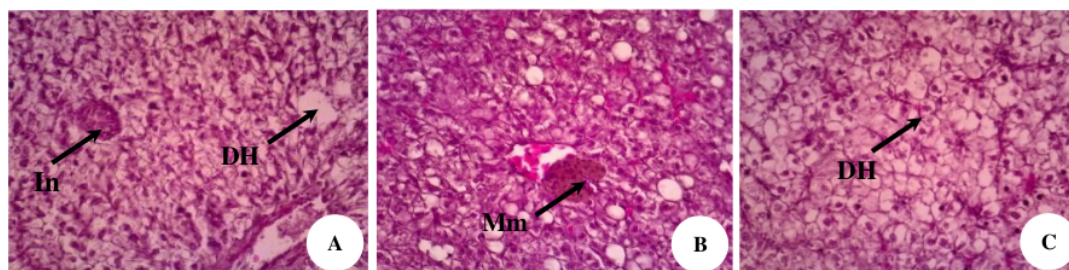


Figure 2. Histopathology of the liver of African catfish (*C. gariepinus*) (400x magnification). The control (C) treatment shows the condition of histological changes in the form of hydropic degeneration (DH) and inflammation infiltration (In). Treatment A shows the condition of histological changes in the form of melanomacrophage (Mm) and treatment B shows the conditions of histological changes in the form of hydropic degeneration (DH).

Discussion

Ammonia is derived from leftover feed and feces of fish (Randall and Tsui 2002) or the composition of organic matter by microorganisms (Kir et al. 2016). The effect of *Bacillus* as a probiotic on African catfish rearing media is presented in Table 1. The results showed that the ammonia value increased as the day progresses and the ammonia value in probiotic treatment was better than the control. This result might be related to the role of probiotic in nitrification and denitrification process in the fish water culture. Boyd and Gross (1998) reported that probiotic application increased nitrification and denitrification of organic matter in the water. The addition of *Bacillus* probiotic was reported to decrease the value of ammonia and nitrite in white shrimp rearing (Nimrat et al. 2012). Zokaifar et al. (2014) noted that the administration of *B. subtilis* of 10^8 CFU/mL in the water could reduce concentration of ammonia, nitrite, and nitrate of shrimp culture. The best ammonia value on the 45th day was found in treatment A. Based on these results, the treatment of the rearing media with *Bacillus NP5* of 1×10^9 CFU/mL, could reduce the amount of ammonia dissolved in it. Similarly, Banerjee and Ray (2017) stated that the application of probiotics could eliminate ammonia and nitrite through nitrification in the fish environment. This is in line with the research conducted by Yang et al. (2011), which stated that the *Bacillus* sp. could convert ammonia to nitrogen under

aerobic conditions. A similar result was reported in the previous study that the total ammonia in the rearing media of fish or shrimp, was lower in the one treated with probiotic compared with the control (Banerjee et al. 2010; Nimrat et al. 2011; Devaraja et al. 2013; Mahmud et al. 2016). In addition, the low ammonia in treatment A was believed to be related to the FCR value in this treatment. The results showed that the lowest significant FCR value was found in this treatment. This might be due to good water quality in this treatment so that the value of FCR increases. According to Kir et al. (2019), good water quality is an important factor for fish growth in any aquaculture system, and ammonia is one of the toxic compounds which decreases the quality of water used in fish rearing. This supports the statement of NRC (2011) that low feed conversion values are indications that the feeds were used optimally.

Temperature and pH are factors that affect the activity of bacteria to oxidase ammonium in the water (Esoy et al. 1998; Hastuti 2011). The results also showed that there were no significant differences in temperature and pH among the treatments. This indicates that the ammonia value obtained in this study not related to temperature and pH. Based on the research conducted by Bhatnagar and Devi (2013), the temperature and pH obtained in this research are still within the range needed in catfish rearing. Similar results were also reported by Abdul-El-Atta et al.

(2019) that the addition of *Lactobacillus plantarum* has no effect on temperature, dissolved oxygen, and pH of tilapia rearing media. Gupta et al. (2016) also stated that the application of *B. coagulans* had no effect on the temperature, pH, and DO values of freshwater prawn rearing media. The similar result was observed in the research conducted by Nimrat et al. (2012) on white shrimp (*Litopenaeus vannamei*), Madani et al. (2018) on white shrimp and Elsabagh (2018) on tilapia, that the provision of probiotics did not affect on the temperature of the fish rearing media. Zhou et al. (2009) also reported that the addition of probiotic *B. coagulans* SC8168 had no effect on the pH of larvae shrimp (*Penaeus vannamei*) rearing media.

The results on growth performance and feed conversion showed a significant ($P < 0.05$) increase with the addition of *Bacillus* NP5 in African catfish rearing media for all periods. This is believed to be due to the role of probiotics in converting ammonia in African catfish rearing media, leading to optimal growth. The better growth performance in the treatments with probiotics is believed to be related not only to the environmental conditions but also to the health status (Xiong et al. 2016; Chumpit et al. 2017). Furthermore, the results of this research showed that the amount of feed consumption was not significant ($P > 0.05$) among the treatments for the entire period. These corroborate the results of previous research which stated that the addition of probiotics to rearing media and feed did not affect fish appetite or feed palatability (Nargesi et al. 2019). There was no significant difference ($P > 0.05$) in the survival rate at the initial period (0-30 days). However, the survival rate in the treatment with probiotic was higher compared with the control after the challenge test period with *A. hydrophila* (30-45 days). This is an indication that probiotic *Bacillus* NP5 could improve the immune system of African catfish thereby making it more resistant to bacterial attacks. According to Dawood et al. (2018) and Zorriehzahra et al. (2016), the mechanism of action of probiotics in converting suppressing the growth of pathogenic bacteria is due to its ability to inhibit the colonization of these bacteria by producing bacteriostatic compounds such as bacteriocins, hydrogen peroxide, proteases, and lysozyme. Probiotics increase the immune

response through physiological modulation (Adel et al. 2017). Al-Hisnawati et al. (2017) also reported that the administration of *Pediococcus acidilactici* MA18/5M as probiotic can modulate the microbiota and increase the innate immune response of rainbow trout. Similar results were reported in several studies that the addition of probiotics increased the survival rate and immune response of fish after challenge test, such as in juvenile white shrimp, *L. vannamei* against *Vibrio harveyi* infection (Zokaeifar et al. 2014), freshwater prawn infected with *Vibrio harveyi* (Gupta et al. 2016), Pacific white shrimp against *V. harveyi* (Hamsah et al. 2019) and *V. parahaemolyticus* (Kewcharoen and Srisapoom 2019), hybrid grouper infected by *V. harveyi* (Li et al. 2019), tilapia infected by *Streptococcus agalactiae* (Zhu et al. 2019), Japanese eel against *A. hydrophila* (Park et al. 2019).

The hematological parameters are used to detect physiological changes in fish bodies due to pathogen attack (Al-dohail et al. 2009). In this research, the value of erythrocytes, leukocytes, and phagocyte activity significantly ($P < 0.05$) increased in the treatments with probiotics after *A. hydrophila* infection (45 days). This indicates that catfish in rearing media with probiotics have a better immune response. This result is thought to be related to the role of probiotics in the modulation of innate immune responses. Probiotic can increase innate immune systems such as neutrophils (Hoseinifar et al. 2018), monocytes (Aly et al. 2014), macrophage (Kumar et al. 2008), and leucocytes (Korkea-aho et al. 2012). It corroborates the results of previous research that the addition of probiotic *Bacillus* NP5 could increase the value of erythrocytes, leukocytes, and phagocytic activity in tilapia infected with *Streptococcus agalactiae* (Agung et al. 2015) and *P. hypophthalmus* infected with *A. hydrophila* (Tamamduhuri et al. 2016). The results showed that the value of erythrocytes after the challenge test decreased for all treatments. This is due to *A. hydrophila* infection which produced both exotoxin and endotoxin enzymes leading to the lysis of red blood cells (Zhang et al. 2014). These results are consistent with Agung et al. (2015) who reported that pathogen infection caused a decrease in erythrocyte values in tilapia.

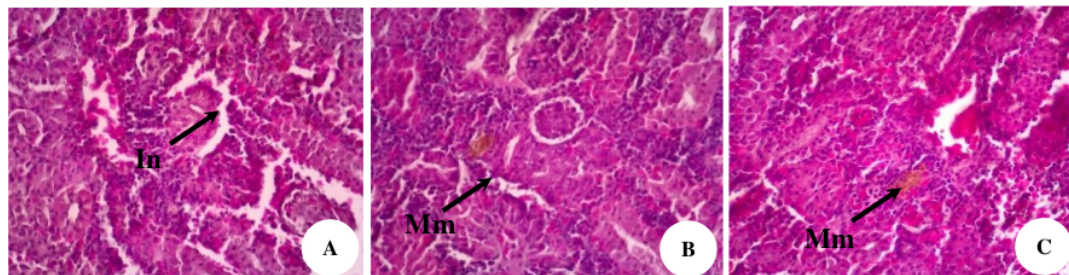


Figure 3. Histopathology of the kidney of African catfish (*C. gariepinus*) (400x magnification). The control (C) treatment shows the condition of histological changes in the form of inflammatory cell infiltration (In). Treatment A and treatment B show the condition of histological changes in the form of melanomacrophage (Mm)

According to Uribe et al. (2011), leukocytes are part of the body defense system which is nonspecific. The results showed that at the end of rearing period, treatment A had the highest significant ($P < 0.05$) leukocytes among the treatments. These indicate that African catfish with probiotic *Bacillus NP5* of 1×10^9 CFU/mL have a better body defense mechanism against pathogen attacks. Similar results were obtained by da Paixão et al. (2017) who reported that the addition of commercial probiotics *B. subtilis* and *Saccharomyces cerevisiae* increased leukocyte values after infection with *Streptococcus agalactiae*. Furthermore, the value of phagocytic activity was higher in the treatments with probiotics. According to Balcazar et al. (2006), phagocytic activity is an inflammatory response which serves as initial resistance before the production of antibody.

Furthermore, the results showed there was damage to the African catfish gill tissue after infection with *A. hydrophila*. This is shown by the tissue changes in the form of hyperplasia in all gills of African catfish in the treatments. Laith and Najiah (2013) stated that the symptoms of *A. hydrophila* attacks are limp fish, decreased appetite, necrosis, and hypertrophy of the skin, hyperplasia and infiltration of leukocytes in the gills, inflammation in the kidneys, liver, and spleen. In addition to hyperplasia, desquamation and telangiectation occur in the gills of African catfish in the control. This telangiectasis occurs in the gills of fish in poor water quality, attacked by pathogens, as well as with accumulation of metabolic and chemical pollutants (Robert 2001). The histology results of the liver showed more histological changes in the control. These changes include hydropic degeneration and inflammation infiltration. However, there was melanomacrophage in treatment A while B had hydropic degeneration. Inflammatory cell infiltration and melanomacrophage occur in the kidneys of African catfish infected with *A. hydrophila*. According to Mokhtar (2017), hydropic degeneration, melanomacrophages, and inflammatory infiltration are all indications of tissue damage due to pathogen infection. The degree of damage to all probiotic treatments only occurs focally. This is believed to be due to the effect of the probiotics in rearing media which only reduces the number of *A. hydrophila* without causing severe organ damage.

Based on the results, it could be concluded that the addition of probiotic *Bacillus NP5* on rearing media significantly improved the growth performance, survival rates, and resistance of African catfish to *A. hydrophila* infection. The degree of tissue damage due to *A. hydrophila* infection in the treatments with probiotics supplementation was smaller compared with that of the control. Also, the probiotic of 1×10^9 CFU/mL (A) significantly resulted in the best SGR and FCR at $2.02 \pm 0.07\%$ day⁻¹ and 1.05 ± 0.07 respectively. Similarly, the highest number of leukocytes at $6.69 \pm 0.17 \times 10^4$ cells/mm³ and phagocyte activity at $65.19 \pm 0.04\%$ were found in treatment A. Furthermore, the addition of probiotics of 1×10^9 CFU/mL resulted in the lowest ammonia value at 1.91 ± 0.17 mg/L. However, the

administration of probiotics did not affect the temperature, DO and pH values of the African catfish rearing media.

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