Original article DOI: 10.19027/jai.23.2.134-146

Application of probiotic microcapsules *Bacillus cereus* BR2 at different doses for the prevention of *Aeromonas hydrophila* NFC1 infection in catfish *Clarias* sp.

Aplikasi mikrokapsul probiotik *Bacillus cereus* BR2 dengan dosis berbeda untuk pencegahan infeksi *Aeromonas hydrophila* NFC1 pada ikan lele *Clarias* sp.

Nurul Faoziyatunnisa[1], Munti Yuhana[2]*, Dinamella Wahjuningrum[2], Sri Nuryati[2], Usamah Afiff[3]

[1]Aquaculture Science, Post Graduate Study Program, Faculty of Fishery and Marine Sciences, IPB University, Darmaga Campus, Bogor, West Java, Indonesia 16680
[2]Department of Aquaculture, Faculty of Fisheries and Marine Science, IPB University, Bogor, West Java 16680, Indonesia
[3]Division of Veterinary Disease and Public Health, Veterinary and Biomedical School, IPB University, Bogor, West Java, Indonesia
*Corresponding author: muntiyu@apps.ipb.ac.id

(Received August 23, 2023; Received in revised form September 27, 2023; Accepted October 31, 2023)

ABSTRACT

This study aims to analyze the administration of microencapsulated probiotic *Bacillus cereus* BR2 through feed atdifferent doses on the survival, immune response, and digestive enzyme activity of catfish *Clarias* sp. infected with *Aeromonas hydrophila* NFC1. The research design consisted of five treatments and three replications, (K-) feeding without the supplementation of probiotics fish injected with Phosphate-buffered saline solution, (K+) feeding without the supplementation of probiotics fish injected with *A. hydrophila* NFC1 cell suspension (10^6 CFU/mL), (P1) feeding with supplementation of *B. cereus* BR2 microcapsules at a dose of 0.5% (w/w), (P2) feeding with the supplementation of *B. cereus* BR2 microcapsules at a dose of 1% (w/w; (P3) feeding with the supplementation of *B. cereus* BR2 microcapsules at a dose of 2% (w/w). All treatments of P1, P2, and P3 were intramuscularly injected with *A. hydrophila* NFC1 cell suspension. Catfish in average body weight of 3.73 ± 0.22 g were reared in tanks filled with 30 liters of water, at a density of 15 fish per container. A feed supplementation trial was performed for 40 days with feeding times three times a day and a 5% feeding rate reevaluated based on biomass. Fish rearing was continued after the challenge test with A. hydrophila NFC1 for 10 days. The results showed that the application of supplemented feed containing *B. cereus* BR2 probiotic microcapsules increased the survival rate, immune response, and digestive enzyme activities of catfish which was infected with *A. hydrophila* NFC1, 2% microcapsule supplementation demonstrated the best result.

Keywords: *Aeromonas hydrophila* NFC1, *Bacillus cereus* BR2, catfish, microcapsule, probiotics

ABSTRAK

Penelitian ini bertujuan untuk menganalisis pemberian mikrokapsul probiotik *Bacillus cereus* BR2 melalui pakan dengan dosis berbeda terhadap sintasan, respons imun, dan aktivitas enzim pencernaan ikan lele *Clarias* sp. yang diinfeksi *Aeromonas hydrophila* NFC1. Rancangan penelitian yang digunakan terdiri dari lima perlakuan dan tiga ulangan, yaitu (K-) pemberian pakan tanpa suplementasi probiotik dan diinjeksi PBS, (K+) pemberian pakan tanpa suplementasi probiotik dan diinfeksi *A. hydrophila* NFC1, (P1) pemberian pakan dengan suplementasi mikrokapsul *B. cereus* BR2 dosis 0,5% (b/b), (P2) pemberian pakan dengan suplementasi mikrokapsul *B. cereus* BR2 dosis 1%, (P3) pemberian pakan dengan suplementasi mikrokapsul *B. cereus* BR2 dosis 2%, masing-masing diinfeksi *A. hydrophila* NFC1. Benih lele dengan ukuran bobot rata-rata sekitar 3,73 ± 0,22 g dipelihara di dalam akuarium bervolume 30 L dengan kepadatan 15 ekor per wadah selama 40 hari, dengan pemberian pakan bersuplemen sebanyak tiga kali sehari, dengan 5% pemberian pakan yang dievaluasi berdasarkan bobot biomassa. Pemeliharaan ikan dilanjutkan setelah uji tantang dengan *A. hydrophila* NFC1 (10^6 CFU/mL) yang dilakukan hingga 10 hari pasca injeksi. Hasil penelitian menunjukkan aplikasi mikrokapsul probiotik *B. cereus* BR2 melalui pakan mampu meningkatkan sintasan, respons imun, dan aktivitas enzim pencernaan ikan lele yang diinfeksi *A. hydrophila* NFC1, dengan suplementasi mikrokapsul 2% sebagai hasil terbaik.

Kata kunci: *Aeromonas hydrophila* NFC1, *Bacillus cereus* BR2, ikan lele, mikrokapsul, probiotik

INTRODUCTION

Catfish (*Clarias* sp.) is one of the popular freshwater fish commodities in Indonesia because of its economical price (Fitria & Nugroho, 2023), shorter cultivation time periods compared to other freshwater commodities (Fauziah *et al*., 2016), as well as its high nutritional values (Apriyana, 2014). Catfish have advantages in terms of environmental aspects, namely that they can be cultivated in a narrow ponds or tanks but cultivable in higher stocking density with a minimum water exchange and in various types of containers (Su'udi & Wathon, 2018). Catfish can be stocked at a high density of 150-400 fish/m^3 of water (Krisnando & Sujarwanta, 2014). Easy and relatively short rearing duration causes many fish farmers to choose catfish farming (Rabilla *et al*., 2018).

Catfish farming production in Indonesia has fluctuated over the last five years. Catfish production in 2017, 2018, 2019 and 2020 were 1,101,232 tons, 944,779 tons, 1,088,945 tons, and 993,768 tons, respectively, while in 2021 it increased to 1,253,114 tons, respectively (KKP, 2021). Catfish production in Bogor Regency in 2018 was 88,781 tons, in 2019 it was 89,916 tons, while in 2020 it decreased to 85,490 tons (BPS, 2021). Infectious disease is one of the main problems causes the production loss in catfish. The negative interaction between fish, pathogens and mismatched environmental conditions will cause fish stress and cause disease (Snieszko, 1974).

One of the serious problems faced by catfish farmers is fish disease caused by bacterial infection (Cerlina *et al*., 2021). Examples of pathogenic bacteria in African catfish are *Edwardsiella tarda, Plesiomonas shigelloides, Alcaligenes faecalis, A. hydrophila* and *A. caviae* (Yuliantoro *et al*., 2017). *A. hydrophila* is a pathogenic bacterial strain that has the most influence in freshwater aquaculture because it can cause 80% to 100% fish mortality in a short time, namely one to two weeks (Fitria *et al*., 2021). Probiotics are live microbes with benefit effects to the host can assist digestion process in gastrointestinal tract and improved immunity against pathogenic bacteria.

The function of probiotics is as mucosal defense, for protection and defense of gastrointestinal immunity (Yonata & Farid, 2016; Fidyandini *et al*., 2016). The productivity of fish farming can be increased by probiotics application, because its function as an immunomodulator and improve the mechanism of the feed digestion process in fish (Wanka *et al*., 2018). Probiotics administration can increase optimal fish growth and fish are not susceptible to disease due to environmental stress (Soeprapto *et al*., 2022). The weakness of probiotic bacteria is that they cannot maintain cell viability during processing, long duration of storage, distribution, and consumption (Hanidah *et al*., 2021). The probiotic microencapsulation process is expected to be able to overcome these problems (Widanarni *et al*., 2023; Febrianti *et al*., 2016; Agung *et al*., 2016). Microencapsulation is a technique that aims to provide probiotic cells with a physical barrier to maintain the survival of probiotic bacteria during processing and storage (Das *et al*., 2014; Yunarty *et al*., 2016; Rosenberg *et al.,* 2016).

Microencapsulation of probiotics aims to increase shelf life, change the form for easier use, handling, and packaging (Kailasapathy, 2002), and increase the viability and stability of probiotics (Das *et al*., 2014). The microencapsulation process of probiotics can be done in several ways, namely extrusion, emulsion, *freeze drying,* and *spray drying* techniques (Setiarto *et al*., 2018). The commonly used microencapsulation technique is spray drying because it has several advantages, namely economical, easy to apply, able to produce microcapsules in large quantities and effective to protect the core material (Poshadri & Kuna, 2010; Hasrini *et al*., 2017). This is what underlies the administration of microencapsulated probiotic *Bacillus cereus* BR2 as feed supplementation for improvement the survival, immune response, and digestive enzyme activity of catfish *Clarias* sp. against MAS.

MATERIALS AND METHODS

Research design

The study consisted of five treatments and three replicates, namely (K-) feeding without the supplementation of probiotic microcapsules and injected with *Phosphate Buffered Saline* (PBS), (K+) feeding without the supplementation of probiotic microcapsules and injected with *A. hydrophila* NFC1, (P1) feeding with the supplementation of 0.5% probiotic microcapsules *B. cereus* BR2 and injected with *A. hydrophila* NFC1, (P2) feeding with the supplementation of 1% probiotic microcapsules *B. cereus* BR2 and injected with *A. hydrophila* NFC1, (P3) feeding

with the supplementation of 2% microcapsules of probiotic *B. cereus* BR2 and injected with *A. hydrophila* NFC1.

Preparation of containers and rearing media

The container used were 15 aquarium units measuring 60×30×25 cm^3 with a water volume of 30 L. Aquariums that will be used washed first using soap and then rinsed using water until clean. The aquarium was filled with fresh water and then added chlorine as much as 30 mg/L, and strongly aerated for 24 hours. Furthermore, the aquarium was added with thiosulfate as much as 60 mg/L, and vigorously aerated again for 24 hours.

Preparation and culture of experimental fish

The fish used were catfish (*Clarias* sp.) with a weight of 3.73 ± 0.22 g and a length of 7.6 ± 0.41 cm. Catfish were first acclimatized for seven days in a tank. Catfish that have gone through the acclimatization process are then placed into an aquarium with a density of 15 fish of each aquarium. The duration of rearing of catfish with different treatments was 40 days, followed by a challenge test with *A. hydrophila* NFC1 cells and further rearing for 10 days post infection.

Preparation of microcapsules of probiotic *B. cereus* BR2

The probiotic used was *B. cereus* BR2 bacterial isolate from the IPB Aquatic Organism Health Laboratory, which was isolated from traditional shrimp ponds area. The *B. cereus* BR2 bacteria were marked as rifampicin antibiotic resistant (BR2 Rf^R). The production of probiotic biomass BR2 Rf^R to be microencapsulated was carried out by culturing on 50 mL Trypticase Soya Broth (TSB) media. Probiotic bacteria BR2 Rf^R were re-cultured in 500 mL of TSB media on a shaker for 24 hours at 1400 rpm.

The probiotic cells obtained were homogenized in 500 mL of sterile distilled water containing 10% coating material. The coating materials used were maltodextrin and whey (1:1). The bacterial culture and the coating material were homogenized with a mixer and then microencapsulated with a BUCHI mini spray dryer with an inlet temperature of 131°C-133°C and an outlet temperature of 65°C-70°C. The obtained product of BR2 Rf^R microcapsules were stored into sterile glass bottles and stored at 4°C-8°C (Zubaidah *et al.*, 2015).

Preparation and feeding of supplemented feed

The feed used was commercial feed with 30% protein content. The preparation for test feed supplement of *B. cereus* BR2 Rf^R microcapsules using each different doses, namely 0.5% (w/w), 1% (w/w), and 2% (w/w). The supplement microcapsule powder was then mixed with egg white as much as 2 mL (2%) as the binder, and distilled water as much as 6 mL (6%) for each 100 grams of feed. The feeding rate was 5% based on total fish biomass with the frequency of feeding three times daily at 08.00, 12.00, and 17.00 WIB.

Challenge test

The pathogenic bacteria used for the challenge test was *A. hydrophila* NFC1, a collection from the IPB Aquatic Organism Health Laboratory, which was isolated from one of the intensive breeding ponds of African catfish (*Clarias gariepinus*) from Bogor Regency. The challenge test was conducted after the fish were fed the treatment diet for 40 days of rearing. Fish were challenged by intramuscularly by injecting 0.1 mL of *A. hydrophila* NFC1 suspension cells with density obtained from the LD_{50} test. During the challenge test, fish were fed with commercial feed based on the biomass three times a day at 08.00, 12.00, and 17.00 WIB. Fish rearing after the challenge test was carried out for 10 days.

Test parameters

Fish immune response

Calculation of catfish survival rate (Huisman, 1987), hematological tests performed were total erythrocytes and total leucocytes (Hesser, 1960), hematocrit levels and phagocytosis activity (Anderson & Siwicki, 1995), hemoglobin levels (Gillet *et al.*, 2009), and respiratory burst (Divyagnaneswari *et al.*, 2007).

Digestive enzyme activity

Digestive enzyme activity parameters were including protease (Walter, 1984), amylase (Bernfeld, 1995), and lipase (Yanbo & Zirong, 2006).

Probiotics and pathogenic bacterial monitoring

Bacterial counts conducted were probiotic bacteria of *B. cereus* BR2 in microcapsule product, intestinal probiotic bacteria of *B. cereus* BR2, and pathogenic bacteria of *A. hydrophila* NFC1 in the target organs of kidney and liver. All

parameters were conducted using the total plate count (TPC) method (Barus *et al*., 2017).

Data analysis

Data from measurements in this study were tabulated using Microsoft Excel 2019 software. Parameter data were subjected to normality and homogeneity tests (P 0.05), then the data were statistically analyzed using variance (ANOVA) with a 95% confidence interval using SPSS 26 software. If the data shows a significant difference (P 0.05), a further test is carried out using the Duncan test.

RESULTS AND DISCUSSION

Results

Fish Survival rate

The catfish' survival rate prior and post challenge test is depicted in Figure 1. Prior challenge test, 100% survival were resulted in all treatment indicated that the fish used in the experiment were in healthy condition and there was no negative impact of the probiotic supplementation. Post challenge test, it is shown that the application of probiotic microcapsules of *B. cereus* BR2 in feed affected the health status and fish survival rate. The survival rate of catfish after the challenged test using *A. hydrophila* NFC1 isolate, the survival rate decreased in each treatment (Figure 1). The survival rate of catfish fed the P1, P2, and P3 treatments were 68.90 ± 3.85%, 82.22 ± 3.85% and 86.67 ± 6.67%, respectively, higher and significantly different ($p<0.05$) from the K+ treatment which was 51.11 ± 3.85%.

Immune response parameters

The catfish' immune response parameters are shown in the following Figures, Figure 2 prior

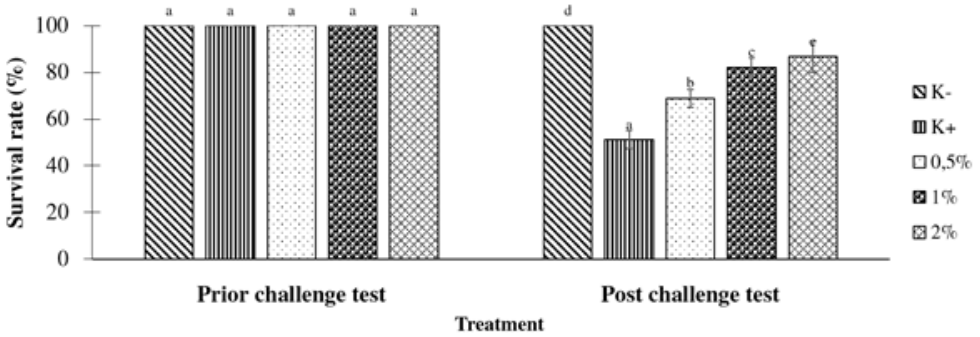

Figure 1. Survival rate parameter of catfish fed with different doses of *B. cereus* BR2 probiotic microcapsules supplementation prior (left) and post (right) challenge test.
The letters above the bars indicate significant differences (LSD, P<0.05). The values presented are mean and standard deviation. Negative control (K-), positive control (K+), 0.5% *B. cereus* BR2 microcapsules (P1), 1% *B. cereus* BR2 microcapsules (P2), 2% *B. cereus* BR2 microcapsules (P3).

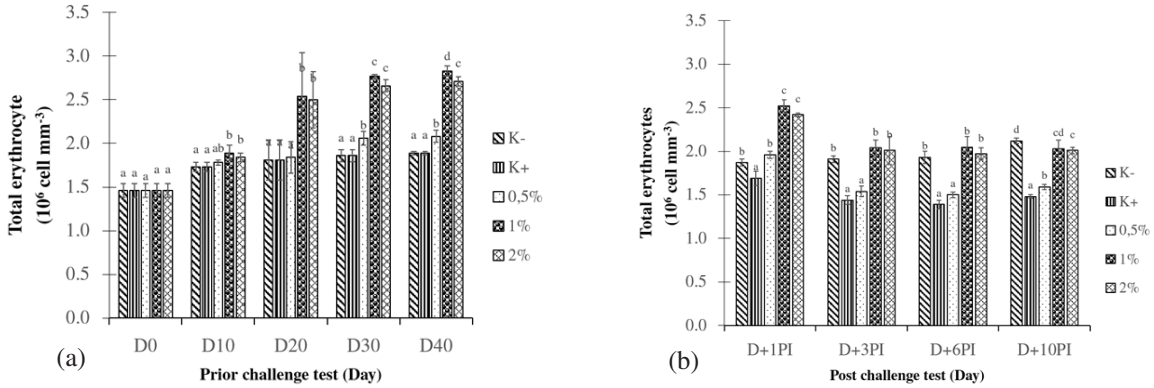

Figure 2. Total erythrocyte parameter of catfish fed with different doses of *B. cereus* BR2 probiotic microcapsules supplementation prior (a) and post (b) challenge test.
The letters above the bars indicate significant differences (LSD, P<0.05). The values presented are mean and standard deviation. Negative control (K-), positive control (K+), 0.5% *B. cereus* BR2 microcapsules (P1), 1% *B. cereus* BR2 microcapsules (P2), 2% *B. cereus* BR2 microcapsules (P3).

and post challenge test of total erythrocytes (TE) parameter, Figure 3 prior and post challenge test of hematocrit (Hc) parameter, Figure 4 prior and post challenge test of hemoglobin (Hb) parameter, Figure 5 prior and post challenge test of total leucocytes (TL), Figure 6 prior and post challenge test of phagocytic activity (PA), and Figure 7 prior and post challenge test of respiratory burst activity (RB). The application of probiotic microcapsules of *B. cereus* BR2 in feed affected the hematological profiles of catfish prior and post challenge test with *A. hydrophila* NFC1. Total erythrocytes (TE), hematocrit (Hc), and hemoglobin (Hb) of catfish prior supplementation diet (D0) were 1.46 ± 0.08 ×10^6 mm/cells^{-3}, 7.38 ± 3.07%, and 4.73 ± 0.23 g/%, respectively. The TE, Hc, and Hb values of catfish continued to increase after feeding the treatment for 40 days.

The best result of the increase in TE value was treatment P2 (2.83 ± 0.06 ×10^6 mm^{-3}/cells) which was significantly different ($p<0.05$) from treatment K (1.89 ± 0.02 ×10^6 mm^{-3}/cells). The best results of the increase in Hc values are P2 (39.82 ± 0.61%) and P3 (37.87 ± 0.58%), which have higher values and significantly different ($p<0.05$) from treatment K (26.70 ± 1.01%). The best results of increasing Hb values were P2 (12.30 ± 0.26 g/%) and P3 (11.83 ± 0.25 g/%) treatments, which had higher values and significantly different ($p<0.05$) from K (9.30 ±

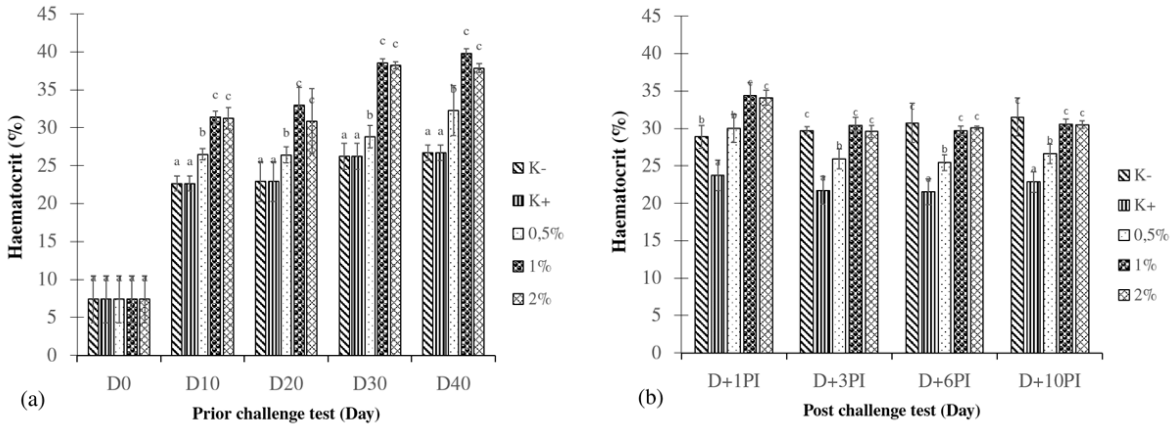

Figure 3. Haematocrit parameter of catfish fed with different doses of *B. cereus* BR2 probiotic microcapsules supplementation prior (a) and post (b) challenge test.
The letters above the bars indicate significant differences (LSD, $P<0.05$). The values presented are mean and standard deviation. Negative control (K-), positive control (K+), 0.5% *B. cereus* BR2 microcapsules (P1), 1% *B. cereus* BR2 microcapsules (P2), 2% *B. cereus* BR2 microcapsules (P3).

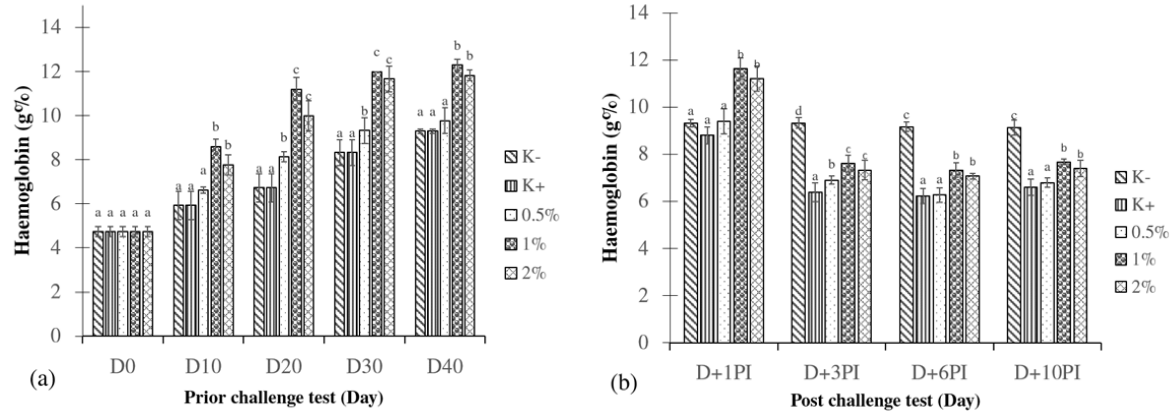

Figure 4. Haemoglobin parameter of catfish fed with different doses of *B. cereus* BR2 probiotic microcapsules supplementation prior (a) and post (b) challenge test.
The letters above the bars indicate significant differences (LSD, $P<0.05$). The values presented are mean and standard deviation. Negative control (K-), positive control (K+), 0.5% *B. cereus* BR2 microcapsules (P1), 1% *B. cereus* BR2 microcapsules (P2), 2% *B. cereus* BR2 microcapsules (P3).

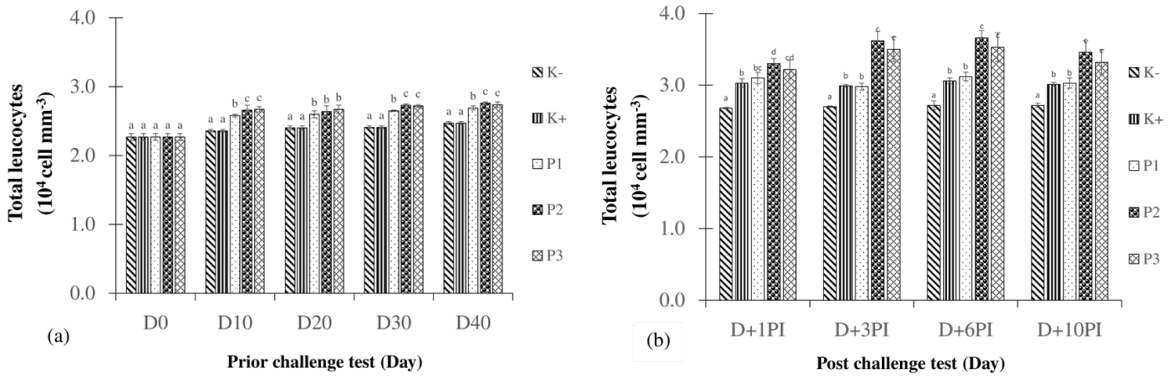

Figure 5. Total leucocyte parameter of catfish fed with different doses of *B. cereus* BR2 probiotic microcapsules supplementation prior (a) and post (b) challenge test.
The letters above the bars indicate significant differences (LSD, P<0.05). The values presented are mean and standard deviation. Negative control (K-), positive control (K+), 0.5% *B. cereus* BR2 microcapsules (P1), 1% *B. cereus* BR2 microcapsules (P2), 2% *B. cereus* BR2 microcapsules (P3).

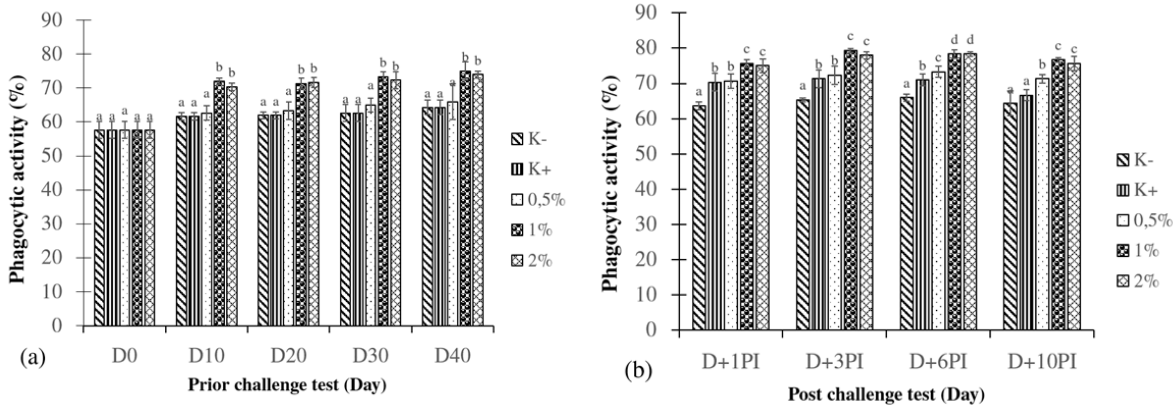

Figure 6. Phagocytic activity parameter of catfish fed with different doses of *B. cereus* BR2 probiotic microcapsules supplementation prior (a) and post (b) challenge test.
The letters above the bars indicate significant differences (LSD, P<0.05). The values presented are mean and standard deviation. Negative control (K-), positive control (K+), 0.5% *B. cereus* BR2 microcapsules (P1), 1% *B. cereus* BR2 microcapsules (P2), 2% *B. cereus* BR2 microcapsules (P3).

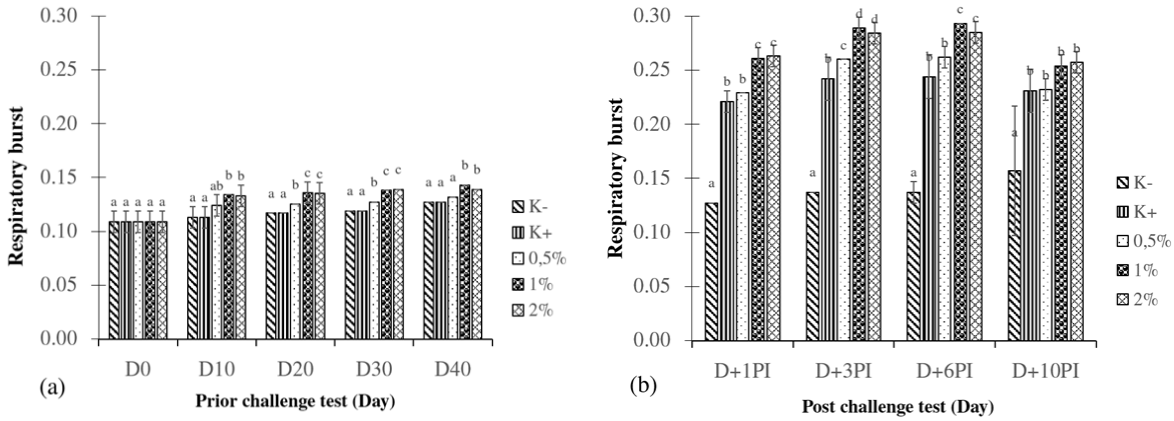

Figure 7. Respiratory burst activity of catfish fed with different doses of *B. cereus* BR2 probiotic microcapsules supplementation prior (a) and post (b) challenge test.
The letters above the bars indicate significant differences (LSD, P<0.05). The values presented are mean and standard deviation. Negative control (K-), positive control (K+), 0.5% *B. cereus* BR2 microcapsules (P1), 1% *B. cereus* BR2 microcapsules (P2), 2% *B. cereus* BR2 microcapsules (P3).

0.10 g/%) and P1 (9.77 ± 0.58 g/%) treatments.

TE, Hc, and Hb values began to decrease from the first day to the sixth day after the catfish were challenged with *A. hydrophila* NFC1. Total leucocytes (TL), phagocytic activity (PA), and respiratory burst activity (RB) of catfish before being fed the treatment diet (D0) were 2.2 7± 0.05 $\times 10^4$ cells/mm^{-3}, 57.67 ± 2.52%, and 0.109 ± 0.01 OD.630nm, respectively. The value of TL, PA, and RB of catfish continues to increase until the sixth day after the challenge test. The best results of the increase in TL values are treatments P2 (3.66 ± 0.10 $\times 10^4$ mm^{-3}/cells) and P3 (3.53 ± 0.20 $\times 10^4$ mm^{-3}/cells) have higher values and significantly different ($p<0.05$) from treatment P1 (3.12 ± 0.06 $\times 10^4$ cells/mm^{-3}), K+ (3.06 ± 0.04 $\times 10^4$ cells/mm^{-3}), and K- (2.72 ± 0.06 $\times 10^4$ cells/mm^{-3}).

The best results of increasing the value of PA are the treatments P2 (78.33 ± 1.16%), P3 (78.33 ± 0.58%), and P1 (73.33 ± 1.53%), which have higher values and significantly different ($p<0.05$) from the treatment K+ (71.00 ± 1.73%). The best results of increasing RB values were P2 (0.29 ± 0.00 OD.630nm) and P3 (0.285 ± 0.01) treatments which were significantly different ($p<0.05$) from the K+ treatment (0.244 ± 0.02). TL, PA, and RB values began to decrease on the 10th day after the challenge test.

Digestive enzyme activity

The activity of digestive enzymes (protease, amylase, lipase) of catfish increased from D0 to the 40th day of feeding treatment (Table 1). The best value of protease enzyme activity is treatment P2 (0.049 ± 0.004 IU/mL) higher and significantly different ($p<0.05$) from treatment K (0.026 ± 0.006 IU/mL). The best value of amylase enzyme activity is treatment P3 (2.33 ± 0.25 IU/mL) higher and significantly different ($p<0.05$) from treatment K (1.58±0.31 IU/mL). The best value of lipase enzyme activity is P3 (0.097 ± 0.006 IU/mL) is higher and significantly different ($p<0.05$) from treatment K (0.019 ± 0.014 IU/mL).

Total bacterial count

The cells population of probiotic *B. cereus* BR2 in microcapsule powder, gastrointestinal of catfish and pathogenic cells of *A. hydrophila* NFC1 in catfish target organs of kidney and intestine are presented in Figure 8 below. The density of microencapsulated *B. cereus* BR2 decreased insignificantly from the first day of microcapsule preparation (11.24 ± 0.23 $\times 10^8$ CFU/g) to the 28th day of microcapsule storage (10.00 ± 0.30 $\times 10^8$ CFU/g). The highest cells density of *B. cereus* BR2 cells in the gut occurred on day 40 of feeding treatment P3 (11.79 ± 0.14 $\times 10^8$ CFU/g). The lowest cells density of *A. hydrophila* NFC1 occurred on the 10th day after the challenge test, namely in the kidney (5.58 ±0.11 $\times 10^6$ CFU/g), and liver P3 (6.21 ± 0.29 $\times 10^6$ CFU/g) which had significantly different values ($p<0.05$) from the positive control treatment.

Discussion

The survival rate of catfish tested using *A. hydrophila* NFC1 and fed with the supplementation of *B. cereus* BR2 probiotic microcapsules has a higher value compared to the control treatment. This result is in accordance with Lusiastuti *et al*. (2017), *Clarias gariepinus* catfish that were challenged with *A. hydrophila* and fed with microencapsulated probiotic supplementation treatment had a survival rate of 97.33%, while with probiotic administration without microencapsulation of 89.33%. Total erythrocytes of catfish decreased from the first day to the sixth day after the challenge test using *A. hydrophila* NFC1. Significant hematological changes in fish can be caused by *A. hydrophila*

Table 1. Digestive enzyme activities of catfish before and after feeding the treatments for 40 days.

Treatments	Protease (IU/mL)		Amylase (IU/mL)		Lipase (IU/mL)	
	D0	D40	D0	D40	D0	D40
K-	0.011 ± 0.003^a	0.026 ± 0.006^a	1.06 ± 0.08^a	1.58 ± 0.31^a	0.014 ± 0.003^a	0.019 ± 0.014^a
K+		0.026 ± 0.006^a		1.58 ± 0.31^a		0.019 ± 0.014^a
P1		0.034 ± 0.009^{ab}		1.94 ± 0.24^{ab}		0.035 ± 0.004^{ab}
P2		0.049 ± 0.004^c		2.07 ± 0.52^{ab}		0.047 ± 0.015^b
P3		0.042 ± 0.004^{bc}		2.33 ± 0.25^b		0.097 ± 0.006^c

Note: Different superscript letters in the same row indicate significant differences (LSD, $P<0.05$). Values presented are mean and standard deviation. Negative control (K-), positive control (K+), 0.5% *B. cereus* BR2 microcapsules (P1), 1% *B. cereus* BR2 microcapsules (P2), 2% *B. cereus* BR2 microcapsules (P3).

infection (Yu *et al*., 2015). The decrease in total catfish erythrocytes after the challenge test was caused by the hemolysin enzyme produced by *A. hydrophila* bacteria (Salosso *et al*., 2020).

The normal range of total erythrocytes of dumbo catfish ranged from two up to three $\times 10^6$ cells/mm^{-3} (Hastuti & Subandiyono, 2015). Catfish blood analysis before being fed the treatment (D0) were anemic because they have low hematocrit levels. According to Nursidah and Putri (2020) one important sign of fish anemia showed by hematocrit level, which is below 22%. Symptoms of anemia in catfish at D0 didn't affect the readiness of the test fish because during 40 days of rearing no dead fish were found, catfish hematocrit levels have also increased since the 10th day of treatment feeding. An increase in hematocrit levels is influenced by an increase in the number of red blood cells, this is because hematocrit is the percent volume of red blood cells in the blood (Yuniastutik, 2021).

The increase in total erythrocytes, hematocrit and hemoglobin levels in catfish was influenced by feeding supplementation with probiotic microcapsules for 40 days, this is in accordance with Setiyaningsih *et al*. (2017) which stated that increasing the dose and frequency of probiotic administration in feed had an effect on increasing the hematocrit and hemoglobin levels of catfish. Hemoglobin is a protein that contains iron in red blood cells and functions to transport oxygen from the lungs to the rest of the body (Fitriany & Saputri, 2018). The decrease in catfish hemoglobin levels after being challenged with *A. hydrophila* NFC1 bacteria is due to the presence of the enzyme hemolysin. The decrease in hemoglobin levels is related to a decrease in the number of red blood cells and hematocrit levels (Astuti & Kulsum, 2020).

The hemolysin enzyme can lyse red blood cells and then cause bleeding and swelling in the fish's body (Hidayat *et al*., 2023). Hemolysin

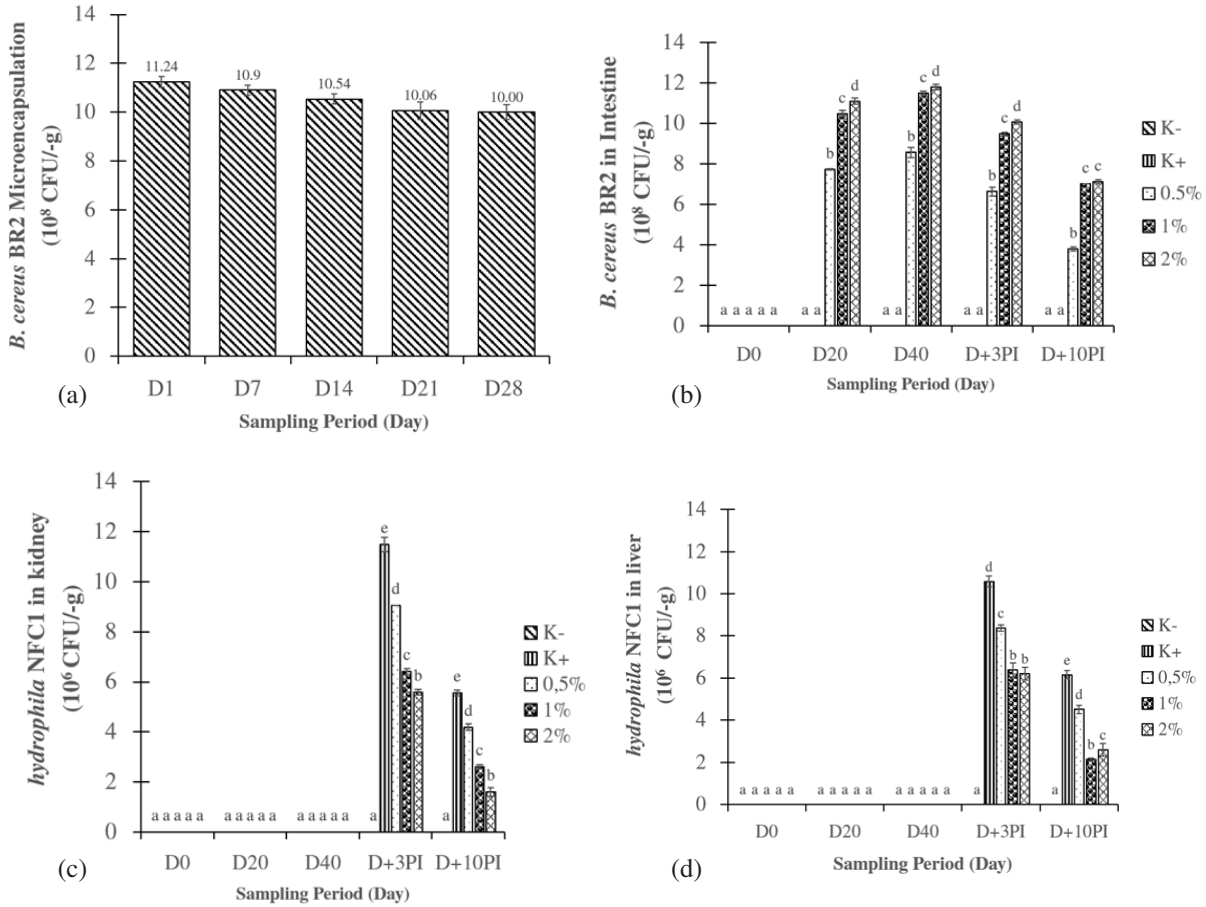

Figure 8. Population of *B. cereus* BR2 in microcapsule product, (a) population of *B. cereus* BR2 in intestine (b) population of *A. hydrophila* NFC1 in target organs: kidney (c) liver (d) catfish. Different letters above the bars indicate significant differences (LSD, P<0.05). Values presented are mean and standard deviation. Negative control (K-), positive control (K+), 0.5% *B. cereus* BR2 microcapsules (P1), 1% *B. cereus* BR2 microcapsules (P2), 2% *B. cereus* BR2 microcapsules (P3).

toxin plays a role in breaking down red blood cells which causes the cells to come out of the blood vessels and causes a reddish color on the surface of the fish's skin. Hemolysin toxin can break down various tissue cells in the fish's body, causing the tissue cells to open (ulcer) (Hermawan *et al*., 2022). Total leucocytes of catfish are still within the normal range of healthy fish which is 2-15 $\times 10^4$ cells/mm^{-3} (Purwanti *et al*., 2014). Leucocyte production in fish given immunostimulants will increase compared to those without immunostimulants, which modulate the fish immunity (Dangeubun & Metungun, 2017).

The increase in white blood cell values, phagocytic activity and respiratory burst after 40 days of treatment feed was due to the presence of probiotics which are immunogenic but the fish's body recognizes them as foreign objects so they can activate immune cells (Djauhari *et al*., 2016). The walls of probiotic bacteria have components in the form of lipopolysaccharide, peptidoglycan and β-glucan which function to stimulate the fish's immune response (Gafur *et al*., 2017). Talpur *et al*. (2014) stated that the leukocytes of *Channa stirata* given probiotic *L. acidophilus* increased after being challenged with *A. hydrophila*. Catfish white blood cells that have been challenged will go to the infected area to prevent virulence (Triyaningsih *et al*., 2014).

The most significant increase in respiratory burst began to occur after the catfish were challenged. This is due to the fish body forming an antibacterial system through respiratory burst activity. Respiratory burst of catfish fed with probiotic microcapsule supplementation of *Bacillus* sp. NP5 and prebiotic mannan oligosaccharide increased after being challenged with *A. hydrophila* (Tamamdusturi *et al*., 2016). The increase in respiratory burst positively synergized with the increase in leucocytes and phagocytosis activity. Probiotic *Bacillus* sp. NP5 can trigger the activity of catfish phagocyte cells to carry out the phagocytosis process against antigens in the form of *A. hydrophila* (Sudrajat *et al*., 2023).

Leukocytes have a role in fish immune response through phagocytosis activity. The results of respiratory burst activity are anions H_2O_2 and superoxide OH^- which can increase the ability of phagocytic cells, and are highly toxic to pathogenic bacteria (Rawling *et al*., 2012). The effectiveness of enzymes in digesting the supplemented feed is influenced by the availability of digestive enzymes, which will then affect the growth (Putri *et al*., 2016). Probiotic bacteria produce exogenous enzymes that can help fish digest feed that enters the digestive tract. The collaboration between exogenous and endogenous enzymes produced by fish can make it easier for feed to be digested and absorbed by fish so that it can improve fish growth performance (Afrilasari *et al*., 2017). The combination of probiotic *Bacillus* sp. NP5 and prebiotic potato extract was shown to increase the activity of catfish digestive enzymes, thus affecting the growth and amount of feed consumption (Putra *et al*., 2020).

Administration of the probiotic *B. megaterium* PTB 1.4 can increase the activity of catfish protease and amylase enzymes (Hamka *et al*., 2020). The population of microencapsulated *B. cereus* BR2 probiotic bacteria decreased insignificantly from the first day of microcapsule preparation to the 28th day the microcapsule product was stored (Figure 8a). The microencapsulation technique is able to maintain the viability of probiotics, and has been proven to reduce the amount of probiotic decline (Trimudita & Djaenudin, 2021). The population of *B. cereus* BR2 probiotic bacteria in the gut increased after 40 days of probiotic supplementation. Probiotic bacteria have the ability to colonize and dominate in the digestive tract of fish (Sumartini *et al*., 2018).

The population of *B. cereus* BR2 probiotic bacteria decreased after the catfish were challenged with *A. hydrophila* NFC1, while in the control treatment no *B. cereus* BR2 bacteria were found (Figure 8b). The population of pathogenic bacteria *A. hydrophila* NFC1 in the kidney and liver of catfish was less than the total probiotic *B. cereus* BR2 in the intestine. This result is in accordance with the statement of Agustin *et al*. (2014), addition of probiotic *Lactobacillus* sp. can reduce the population of the pathogenic bacteria *Aeromonas* sp. and *Pseudomonas* sp. on snakehead fry. The density of *Bacillus* sp. bacteria can dominate the digestive tract compared to pathogenic bacteria (Sun *et al*., 2020).

CONCLUSIONS

Application of probiotic *B. cereus* BR2 microcapsules through feed was able to improve survival, immune response, and digestive enzyme activity of *Clarias* sp. catfish infected with *A. hydrophila* NFC1, with the best results at 2% microcapsule dose.

REFERENCES

Afrilasari W, Widanarni, Meryandini A. 2017. Effect of probiotic *Bacillus megaterium* PTB 1.4 on the population of intestinal microflora, digestive enzyme activity and the growth of catfish (*Clarias* sp.). Hayati Journal of Biosciences 23: 168–172.

Agung LA, Widanarni, Yuhana, M. 2016. Application of microencapsulated probiotic *Bacillus* sp. NP5 and prebiotic mannan oligosaccharide (MOS) to prevent streptococcosis on tilapia *Oreochromis niloticus*. Research Journal of Microbiology 10: 571–581.

Agustin R, Sasanti AD, Yulisman. 2014. Feed conversion, growth rate, survival rate and bacterial populations snakehead fry (*Channa striata*) fed with probiotic. Jurnal Akuakultur Rawa Indonesia 2: 55–66.

Anderson DP, Siwicki AK. 1995. Basic Hematology and Serology for Fish Health Programs. Thailand: Phuket.

Apriyana I. 2014. Effect of adding catfish head flour (*Clarias* sp.) in making cilok on protein content and organoleptic properties. Unnes Journal of Public Health 3: 1–9.

Astuti D, Kulsum U. 2020. Pola menstruasi dengan terjadinya anemia pada remaja putri. Jurnal Ilmu Keperawatan dan Kebidanan 11: 314–327. (In Indonesia).

Barus JG, Santosa PE, Septianova D. 2017. Effect of soaking time using bay leaves (*Szygium polynathum*) as preservative on Total Plate Count and salmonella of broiler meat. Journal of Livestock Research and Innovatio. 1: 42–47.

Bernfeld P. 1955. Amylases α and β. Methods in Enzymology 1: 149–158.

[BPS] Badan Pusat Statistik. 2021. Produksi Ikan Konsumsi Menurut Jenis Ikan (Ton), 2018-2020 https://bogorkab.bps.go.id/indicator/56/72/1/produksi-ikan-konsumsi-menurut-jenis-ikan.html. [5 September 2021].

Cerlina M, Riauwaty M, Syawal H. 2021. Description of Erythrocyte of Clarias gariepinus Infected by Aeromonas hydrophila and Treated with Bay Leaf Extract (Syzygium polyantha). Journal of Fisheries and Marine 27: 105–113.

Dangeubun JL, Metungun J. 2017. Hematology of *Vibrio alginolyticus*-infected humpback grouper *Cromileptes altivelis*, under treatment of *Alstonia acuminata* shoot extract. Aquaculture, Aquarium, Conservation & Legislation 10: 274–284.

Das A, Ray S, Raychaudhuri UR, Chakraborty R. 2014. Microencapsulation of probiotic bacteria and its potential application in food technology. International Journal of Agriculture, Environment & Biotechnology 7: 47–53.

Divyagnaneswari M, Christybapita D, Dinakaran MR. 2007. Enhancement of non-specific immunity and disease resistance in *Oreochromis mossambicus* by *Solanum trilobtaum* leaf fractions. Fish and Shellfish Immunology 23: 249–253.

Djauhari R, Widanarni, Sukenda, Suprayudi MA, Zairin MJ. 2016. Characterization of *Bacillus* sp. NP5 and its application as probiotic for cammon carp (*Cyprinus carpio*). Journal of Microbiology 1: 101–111.

Fauziah AF, Agustina T, Hariyati Y. 2016. Analisis pendapatan dan pemasaran ikan lele dumbodi Desa Mojomulyo Kecamatan Puger. Jurnal Sosial Ekonomi Pertanian 9: 20–32. (In Indonesia).

Febrianti D, Yuhana M, Widanarni. 2016. Dietary synbiotic microcapsule influence the immune responses, growth performance and microbial populations to W*hite Spot Syndrome Virus* in Pacific white shrimp (*Litopenaeus vannamei*). Journal of Fisheries and Aquatic Science 11: 28–42.

Fidyandini HP, Yuhana M, Lusiastuti AM. 2016. Addition of multispecies probiotics in the culture medium of African catfish to prevent the *Motile Aeromonads Septicemia* disease. Jurnal Veteriner 17: 430–439.

Fitria DM, Sukenda S, Yuhana M. 2021. Development of a co-agglutination method for detection of *Aeromonas hydrophila* as causative agent of *Motile Aeromonas Septicemia* (MAS) disease in gourami (*Osphronemus goramy*). Iranian Journal of Fisheries Sciences 20: 141–156.

Fitria D, Nugroho Y. 2023. The production cost of catfish cultivation at the workshop of young farmers in Langkak Village using the ABC method. Jurnal Pertanian Agros 25: 31–41.

Fitriany J, Saputri AI. 2018. Iron deficiency anemia. Malikussaleh Journal of Medicine and Health 4: 1–14.

Gafur A, Jamal K, Hasmawati, Syaichudin M. 2017. Pengujian aplikasi probiotik powder dalam memperbaiki performa benih bandeng

(*Chanos chanos* Forskal) YANG berkualitas. Jurnal Perekayasaan Budidaya Air Payau 2: 95–101. (In Indonesia).

Gillet P, Boei L, Jacobs J. 2009. Practical Note: Tropical Hematology. Antwerp (BE): Prince Leopold Institute of Tropical Medicine.

Hamka MS, Meryandini A, Widanarni. 2020. Growth performance and immune response of catfish *Clarias* sp. given probiotics *Bacillus megaterium* PTB 1.4 and *Pediococcus pentosaceus* E2211. Jurnal Akuakultur Indonesia. 19: 50–60.

Hanidah I, Kirana AI, Nurhadi B, Sumanti DM. 2021. The functionality of probiotic bacteria microencapsulation by spray drying: A Literature Review. Jurnal Teknologi dan Manajemen Agroindustri 10: 274–282.

Hasrini RF, Zakaria FR, Adawiyah DR, Suparto IH. 2017. Microencapsulation of crude palm oil using maltodextrin and soy protein isolate. Journal of Food Technology and Industry 28: 10–19.

Hastuti S, Subandiyono. 2015. Health condition of african catfish (*Clarias gariepinus,* Burch) reared with biofloc technology. Journal of Fisheries Science and Technology 10: 74–79.

Hermawan R, Kurniawan A, Syarif AF. 2022. Effectivity of Canbat herb from PT. Meddia Herbal to *Aeromonas hydrophila* infection on nile fish (*Oreochromis* sp.). Journal of Aquatropica Asia 7: 34–42.

Hesser EF. 1960. Methods for routine fish haematology. The Progressive-Culturist 22: 164–171.

Hidayat S, Septiani G, Agustina A. 2023. Isolates of lactic acid bacteria to control *Aeromonas hydrophila* in tilapia (*Oreochromis niloticus*). Jurnal Ilmu Perikanan Tropis Nusantara 2: 41–49.

Huisman EA. 1987. *Principles of Fish Production*. Department of Fish Culture and Fisheries. Nedherland (NL): Wageningen Agriculture University.

Kailasapathy K. 2002. Microencapsulation of probiotic bacteria: Technology and potential applications. Current Issues in Intestinal Microbiology 3: 39–48.

Krisnando Y, Sujarwanta A. 2014. Comparison of sangkuriang catfish (*Clarias* sp.) growth between silk worm feeding and pellet feeding as a biology learning resource. Bioeducation 5: 1–9.

Lusiastuti AM, Andriyanto S, Samsudin R. 2017. Effectiveness of microencapsulated probiotic combination through feed for the control of motile *Aeromonads septicemia* disease in catfish *Clarias gariepinus.* Journal of Aquaculture Research 12: 179–186.

[KKP] Kementerian Kelautan dan Perikanan. 2021. Laporan Kinerja Kementerian Kelautan dan Perikanan. [14 June 2022].

Nursidah NF, Putri TW. 2020. The effect of sweet potatoes leaves (*Ipomoea batatas*) on blood profile and survival rate of tilapia (*Orechromis niloticus*). Musamus Fisheries and Marine Journal 3: 63–76.

Poshadri A, Kuna A. 2010. Microencapsulation technology: A review. The Journal of Research ANGRAU 38: 86–102.

Purwanti SC, Suminto, Sudaryono A. 2014. The description of blood profile of catfish (*Clarias gariepinus*) that is fed with a combination of artificial feed and 26 earthworm (*Lambricus rubellus*). Journal of Aquaculture Management and Technology 3: 53–60.

Putra AN, Syamsunarno MASB, Ningrum W. 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*). Biodiversity Journal of Biological Diversity 21: 2566–2575.

Putri IW, Setiawati M, Jusadi D. 2016. Digestive enzymes and growth performance of goldfish, *Cyprinus carpio* Linnaeus, 1758 fed with turmeric flour *Curcuma longa* Linn. Indonesian Journal of Ichthyology 17: 11–20.

Rabilla RR, Satria A, Yuliati LN. 2018. Marketing strategy of organic sangkuriang catfish Surya Kencana farm Bogor. IKM Management 13: 137–142.

Rawling MD, Merrifield DL, Snellgrove DL, Kuhlwein H, Adams A, Davies SJ. 2012. Haemato-immunological and growth response of mirror *carp Cyprinus carpio* fed a tropical earthworm meal in experimental diets. Fish & Shellfish Immunology 32: 1002–1007.

Rosenberg M, Rosenberg Y, Frenkel L. 2016. Microencapsulation of model oil in wall matrices consisting of SPI and maltodextrins. AIMS Agriculture and Food 1: 33–51.

Salosso Y, Sunadji, Rebhung F, Anggrany K. 2020. Application of kefa forst honey as antibacterial in the treatment of common

carp *Cyprinus carpio* infected with bacteria *Aeromonas hydrophila*. Bioflux 13: 984–992.

Setiarto RHB, Kusumaningrum HD, Jenie BSL, Khusniati T. 2018. Technology development of probiotic bacteriamicroencapsulation and it benefit for healthy. Veterinary Journal 4: 574–589.

Setiyaningsih L, Widanarni, Lusiastuti AM, Yuhana M. 2017. Effects of dietary probiotic microcapsules *Bacillus cereus* P22 and *Staphylococcus lentus* L1k on growth performance, immune response, and resistance of African catfish, *Clarias gariepinus* Burchell 1822 infected with *Aeromonas hydrophila.* Jurnal Iktiologi Indonesia 17: 143–154.

Snieszko SF. 1974. The effects of environmental stress on outbreaks of infectious diseases of fishes. Journal of Fish Biology 6: 197–208.

Soeprapto H, Ariadi H, Khasanah K. 2022. Pelatihan pembuatan probiotik herbal bagi kelompok pembudidaya ikan. Jurnal Pengabdian Kepada Masyarakat 1: 1929–1934. (In Indonesia).

Sudrajat RH, Yuhana M, Widanarni, Ekasari J, Afiff U. 2023. Dietary supplementation of *Bacillus* sp. NP5 and dayak onion simplicia powder *Eleutherine bulbosa* (Mill.) Urb. for the prevention of *Aeromonas hydrophila* in catfish *Clarias* sp. Jurnal Akuakultur Indonesia 22: 134–146.

Sumartini I, Widanarni, Yuhana M, Santika A. 2018. Growth performance and immune responses of *Clarias* sp. reared with suplementation of probiotic, prebiotic, and synbiotic. Jurnal Riset Akuakultur 13: 329–336.

Sun S, Su Y, Yu H, Ge X, Zhang C. 2020. Starvation affects the intestinal microbiota structure and the expression of inflammatory-related genes of the juvenile blunt snout bream, *Megalobrama amblycephala.* Aquaculture 517: 1–10.

Su'udi M, Wathon S. 2018. Improving the performance of african catfish (*Clarias gariepinus*, Burch) cultivation in Serut Village, Panti District, Jember Regency, East Java Province. Warta Pengabdian 12: 298–306.

Talpur AD, Munir MB, Mary A, Hashim R. 2014. Dietary probiotics and prebiotics improved food acceptability, growth performance, hematology and immunological parameters and disease resistance against *Aeromonas hydrophila* in snakehead (*Channa striata*) fingerlings. Aquaculture 1: 14–20.

Tamamdusturi R, Widanarni, Yuhana M. 2016. Administration of microencapsulated probiotic *Bacillus* sp. NP5 and prebiotic mannan oligosaccharide for prevention of *Aeromonas hydrophila* infection on *Pangasionodon hypophthalmus*. Journal of Fisheries and Aquatic Science 11: 67–76.

Trimudita RF, Djaenudin. 2021. Enkapsulasi probiotik *Lactobacillus* sp. menggunakan dua tahap proses. Serambi Engineering 6: 1832–1841.

Triyaningsih T, Sarjito S, Prayinto B. 2014. Pathogenicity of *Aeromonas hydrophila* isolated from african catfish (*Clarias gariepinus*) from Boyolali. Journal of Aquaculture Management and Technology 3: 11–17.

Walter HE. 1984. Method with hemoglobin, casein and azocoll as substrate. In: Bergmeyer HU, editor. Methods of Enzymatic Analysis 3rdrd Edition. Weinhem: Verlag Cheime.

Wanka KM, Damerau T, Costas B, Krueger A, Schulz C, Wuertz S. 2018. Isolation and characterization of native probiotics for fish farming. BMC Microbiology 18: 1–13.

Widanarni, Yuhana M, Sukenda. 2023. Probiotic dan Prebiotic: Principles and Applications in Aquaculture. Bogor (ID): IPB Press. ISSN 978-603-467-581-8.

Yanbo W, Zirong X. 2006. Effect of probiotics for common carp *Cyprinus carpio* based on growth performance and digestive enzyme activities. Animal Feed Science and Technology 127: 283–292.

Yonata A, Farid AFM. 2016. The Probiotics Use as Therapy in Diarrhea. Majority 5: 1–5.

Yu J, Koo BH, Kim DH, Kim DW, Park SW. 2015. *Aeromonas sobria* infection in farmed mud loach (*Misgurnus mizolepis*) in Korea, a bacteriological survey. Iranian Journal of Veterinary Research 16: 194–201.

Yunarty, Yuhana M, Widanarni. 2016. Effects of microencapsulated synbiotic administration at different dosages against heavy co-infection of white spot disease (WSD) and *Vibrio harveyi* in Pacific white shrimp (*Litopenaeus vannamei*). Indonesian Journal of Marine Science 21: 169–176.

Yuliantoro B, Helmizuryani H, Elfachmi E. 2017. Diversity of pathogenic bacteria in african

catfish (*Clarias gariepinus*) in several farmers in Palembang City. Fiseries 6: 1–6.

Yuniastutik T. 2021. Observasi waktu maksimal penyimpanan darahterhadap nilai hematokrit ikan lele (*Clarias* sp.). Jurnal Teknologi dan Manajemen Pengelolaan Laboratorium 4: 45–50. (In Indonesia).

Zubaidah A, Yuhana M, Widanarni. 2015. Encapsulated synbiotic dietary supplementation at different dosages to prevent vibriosis in white shrimp, *Litopenaeus vannamei*. Hayati Journal of Biosciences 22: 163–168.