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The Effect of Adding *Bacillus* NP5 to Feed on Growth, Survival Rate, and Protection Against *Aeromonas hydrophila* of Catfish (*Clarias* sp.)

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Abstract. Probiotics are useful organisms in preventing the spread of disease in aquaculture. Therefore this study aims to evaluate the effect of administering *Bacillus* NP5 on the growth, survival rate, and histology of catfish with *Aeromonas hydrophila* infection. To achieve this, catfish (7.75 ± 0.02 g) were reared for 45 days with a density of 20 per tank. Furthermore, the study consisted of 4 treatments and 3 replications, namely K+: 0% *Bacillus* NP5+0.1 mL A. *hydrophila* injection, K-: 0% Bacillus NP5 + without A. *hydrophila* injection, A: 0.3% *Bacillus* NP5, and B: 0.8% *Bacillus* NP5. The results showed that the addition of probiotics significantly increased the growth and survival rate of catfish (P <0.05) compared to K+. The higher value of specific growth rate was found in treatments A and B (0.22 ± 0.03 and 0.23 ± 0.03% day-1, respectively) and the lowest in K+ (0.14 ± 0.03% day-1). Also, the tissue damage in K+ was higher than in the probiotic treatment. Besides, hyperplasia, congestion, and secondary lamella fusion in the gill tissue were found in the K+, while other treatments were found only in hyperplasia. Melanomacrophages occurred in catfish kidney tissue for all treatments, however, hydropic degeneration was found in the control treatment. The addition of Bacillus NP5 in feed resulted in higher growth, survival, and tissue damage compared to K+. Also, the addition of 0.8% Bacillus NP5 resulted in less catfish tissue damage.

Keywords: *Bacillus* NP5, catfish, histology, probiotic.

1. Introduction

Catfish is a freshwater commodity with high economic value and is widely cultivated in Indonesia. However, the density of high in catfish culture has led to a decrease in the environmental quality of the rearing media and an increase in disease infection [1]. One of the most common diseases in catfish farming, especially in the intensive culture systems is Motile Aeromonas Septicemia (MAS) caused by the bacterium *Aeromonas hydrophila*. The clinical symptoms of this bacterium disease are lesions and necrosis of the skin with various sizes, reddish ulcers, hemorrhage, and inflammation of the stomach

lining and fins [2]. MAS also attacks the fish digestive tract, which results in reduced feed efficiency and growth decrease [3]. The use of antibiotics to prevent diseases in aquaculture has long been abandoned because it causes bacterial resistance to antibiotics and leaves residues in the fish environment [4, 5].

Probiotics is an approach to prevent the spread of the pathogen in aquaculture [6]. These are live microbes that benefit the host by stimulating the growth of the microbial population in the digestive tract, increasing feed digestibility, enhancing the immune response to pathogen infection, and improving environmental quality [7]. *Bacillus* NP5 is a probiotic isolated from the digestive tract of tilapia [8], which has a positive effect on increasing the growth performance of its host [9]. Furthermore, the administration of *Bacillus* NP5 has been investigated to improve the immune response of tilapia [10, 11], white shrimp [12], Common carp [13], and *Pangasius* sp. [14]. However, no studies have evaluated the effect of its addition on the growth and tissue histology of catfish. Therefore, this study aims to evaluate the effect of the addition of *Bacillus* NP5 as a probiotic on growth, survival rate, and tissue histology of catfish infected by *A. hydrophila*.

2. Material and Methods

2.1 Preparation of Feed

The probiotics were inoculated into 25 mL of *Tryptic Soy Broth* (TSB) media and incubated for 24 hours with a water bath shaker at 29 °C. Subsequently, they were harvested by centrifugation at a speed of 3000 rpm for 30 minutes and diluted from a density of 10^{11} CFU/mL to 10^{8} CFU/mL. Meanwhile, commercial feed with a protein content of 33.9% was mixed with *Bacillus* NP5 and 2% egg yolk according to the method described by [15]. It was dried for 15 minutes to reduce moisture and then the feed was ready to be fed to the fish.

2.2 Catfish Rearing and Growth Parameters

Twelve round fiberglass tanks with a volume of 60 L and a diameter of 50 cm were used for catfish rearing. The tank was sterilized using freshwater and 30 ppm chlorine for 24 hours. Furthermore, catfish with an average weight of 77.75±0.02 g were reared at a density of 20 fish/35 L and were fasted for 24 hours to eliminate the effect of residual feed. Also, as much as 10% of the water was changed every morning. Feeding was carried out using the satiation method with a frequency of three times daily for 45 days. A completely randomized design was adopted, which consist of four treatments and three replications, namely: K+ (positive control with 0.1 mL *A. hydrophila* injection), K- (negative control without *A. hydrophila* injection), A (0.3% *Bacillus* NP5 with 0.1 mL *A. hydrophila* injection), and B (0.8% *Bacillus* NP5 with 0.1 mL *A. hydrophila* injection).

At the end of the experiment (45 days), the weight and number of fish were calculated to determine their growth and survival rate. The growth parameters in this study consisted of specific growth rate (SGR), feed efficiency (FE), and survival rate (SR), which were in line with [17].

$$\text{SGR (\% day}^{-1}) = \frac{Ln\ Wt - Ln\ Wo}{45\ days} \times 100\% \quad (1)$$

$$\text{FE (\%)} = \frac{(Wt + Wd) - Wo}{I} \times 100\% \quad (2)$$

$$\text{SR (\%)} = \frac{Ft}{Fo} \times 100\% \quad (3)$$

Note:
W_0 = initial biomass of catfish, W_t = final biomass of catfish, Wd = biomass of dead catfish, I = feed intake, F_t = final number of catfish, F_o = initial number of catfish.

2.3 A. Hydrophila Injection and Histopathology

After 20 days, catfish (except for treatment K-) were injected intramuscularly with *A. hydrophila* using a syringe. The dose is 0.1 mL/fish with a density of 10^{6} CFU/mL according to [1]. At the end of the experiment, 5 catfish from each treatment were taken for histological analysis according to the method

described by [18]. This was performed on the catfish gill, liver, and kidney at Histopathology Laboratorium, Station for Investigation of Fish Health and Environment, (SIFHE) Serang, Indonesia.

2.4 Statistical Analysis

The histology of gill, liver, and kidney tissue of catfish was analyzed descriptively and the growth data were analyzed using ANOVA (analysis of variance). Furthermore, the Duncan multiple range tests were adopted to analyze the different data [19]

3. Result and Discussion

3.1 Growth and Survival Rate of Catfish

Probiotics do not have a specific mechanism of action in their host body. However, it maintains a balance of microorganism populations in the digestive tract of the host [20]. The effect of probiotics on the growth and survival rate of catfish is shown in Table 1.

Table 1. Effect of the addition of probiotics on growth and survival rate of catfish*

Parameters**	Probiotic treatments***			
	K+	K-	A	B
SGR (% day^{-1})	0.14±0.03^{a}	0.24±0.01^{b}	0.22±0.03^{b}	0,23±0,03^{b}
FE (%)	40.79±7.78	53.72±16.86	55.20±17.31	54,43±13,23
SR (%)	56.67±7.63^{a}	88.33±5.77^{b}	86.67±3.88^{b}	83,33±2,88^{b}

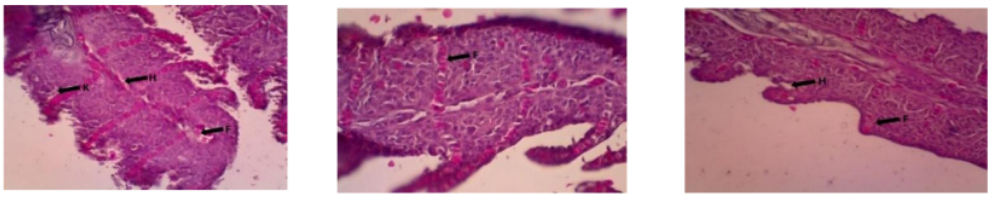
*The same superscript letter on the same line indicates an insignificant value (P>0.05).
**SGR (specific growth rate), FE (feed efficiency), SR (survival rate).
***K+ (positive control with *A. hydrophila* injection), K- (negative control without *A. hydrophila* injection), A (0.3% *Bacillus* NP5 with *A. hydrophila* injection), B (0.8% *Bacillus* NP5 with *A. hydrophila* injection).

The result shows that there is no significant value for the FE parameter between all treatments. Furthermore, the SGR and SR values in probiotic and K- treatments were higher than those of K+ (P<0,05), but there is no significant difference for SGR and SR values between K-, A, and B treatments. Also, the highest SGR was recorded at K- (0,24±0,03 % day^{-1}), followed by B (0,23±0,03 % day^{-1}), A (0,22±0,03 % day^{-1}) and the smallest value was K+ (0,14±0,03 % day^{-1}). Besides, the increased growth in probiotic treatment might be due to increased enzyme activity in the digestive tract of catfish. Hauville [21] reported that the addition of probiotics to feed increases the normal microflora population which was helped in the process of digestion. Also, several studies have shown that the applications of *Bacillus* NP5 as probiotics in the feed increased growth due to the improved activity of enzymes in the digestive tract [9, 22]. Similar results were found by [23] in white shrimp and [24] in snakehead.

The survival rate is a measure of the success of a population in percentage at a given time during fish culture. After *A. hydrophila* infection, the survival rate in probiotic treatments showed a better result than K+. This was an indication that the addition of *Bacillus* NP5 as a probiotic was able to suppress *A. hydrophila* infection. Similarly, [10] reported that the survival rate of tilapia with the administration of *Bacillus* NP5 and infected *streptococcus agalactiae* is 80.56% and 13.89 in the positive control treatment.

3.2 Histology of Catfish Tissue

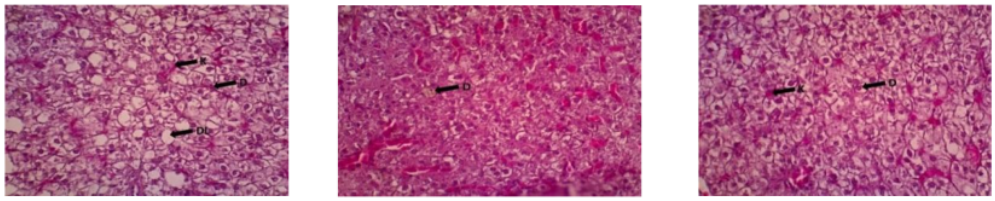
The histology of catfish gill tissue after *A. hydrophila* infection is shown in figure 1. Furthermore, hyperplasia, congestion, and fusion of lamella were found in K+. Also, hyperplasia was found in all treatments except K- and lamella fusion was found in K+, K-, and A. The hydropic degeneration in liver tissue (Figure 2) is found in all treatments and congestion is found in K+ and A. Besides, lipid differentiation was found in K+. Melanomacrophages occur in catfish kidney tissue in all treatments (Figure 3). However, hydropic degeneration was found in K+.



K+ K- A

B

Figure 1. The gill histology of catfish after *A. hydrophila* infection with 400× magnification. K: congestion, H: hyperplasia, F: lamella fusion. K+ (positive control with *A. hydrophila* injection), K- (negative control without *A. hydrophila* injection), A (0.3% *Bacillus* NP5 with *A. hydrophila* injection), B (0.8% *Bacillus* NP5 with *A. hydrophila* injection).

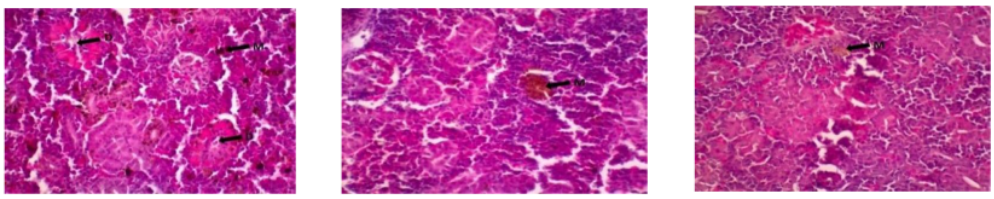


K+ K- A

B

Figure 2. The liver of catfish after *A. hydrophila* infection with 400× magnification. K: congestion, D: hydropic degeneration, DL: lipid differentiation. K+ (positive control with *A. hydrophila* injection), K- (negative control without *A. hydrophila* injection), A (0.3% *Bacillus* NP5 with *A. hydrophila* injection), B (0.8% *Bacillus* NP5 with *A. hydrophila* injection).

Hyperplasia is the most common histopathological change in gills. Meanwhile, the congestion found in K+ is believed to be caused by *A. hydrophila* infection. This result was in line with [25] that congestion was a state of increased blood volumes in the vessels that accumulate in a part of the body due to physical trauma caused by a pathogen or circulatory system disorders. Furthermore, congestion was found in the catfish livers of K+ and lipid degeneration of the liver was characterized by swollen and vacuole tissue. This results in loss of lipid metabolism in fish [26]. In catfish kidneys, the damage was found in the form of melanomacrophages, which are characterized by the presence of inflamed parts and macrophages. Furthermore, tissue damage in probiotic treatment was smaller than K+, which is caused by the role of probiotics in increasing Immune response. A similar result was found by [10] in tilapia and [27] in African catfish.



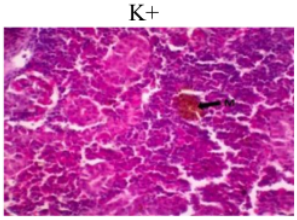


Figure 3. The kidney of catfish after *A. hydrophila* infection with 400× magnification. M: Melanomacrophages, D: hydropic degeneration. K+ (positive control with *A. hydrophila* injection), K- (negative control without *A. hydrophila* injection), A (0.3% *Bacillus* NP5 with *A. hydrophila* injection), B (0.8% *Bacillus* NP5 with *A. hydrophila* injection).

4. Conclusions

Conclusively, the addition of *Bacillus* NP5 as a probiotic increased the growth and survival rate of catfish after *A. hydrophila* infection. Furthermore, tissue damage in probiotic treatment was smaller than K+, besides, there was no significant difference between 0.3% and 0.8 & *Bacillus* NP5 for all parameters.

Acknowledgments

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