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by Sarjito Sarjito

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The performance of survival and immunity of catfish (*Clarias gariepinus*) juvenile infected by *Aeromonas hydrophila* after treated by prebiotic extract in the diet

S B Prayitno, S Riyadi, Sarjito, Desrina

Department of Aquaculture, Faculty of Fisheries and Marine Science, Diponegoro University, Tembalang Campus, Semarang, 50275

corresponding author: sbudiprayitno@gmail.com

Abstract. Herb/plant can act as a prebiotic. Red ginger has long known as an anti-inflammation and anti-oxidant. Similarly, moringa leaf is also known as herbal antibiotic. In the recent years, the use of natural ingredients to stimulate immune system and to combat fish disease in aquaculture becoming a favourable choice. The aims of this research was to observe the red ginger and moringa leaf extract in the diet to the performance of catfish infected by *Aeromonas hydrophila*. Experimental laboratory using a completely randomized design with 6 treatments and 3 replication used in this study. The treatments were mixture of (A) 500 ppm and 0 ppm, (B) 375 ppm and 150 ppm, (C) 250 ppm and 300 ppm, (D) 125 ppm and 450 ppm, (E) 0 ppm and 600 ppm, (F) 0 ppm and 0 ppm mixture of red ginger and moringa leaf extract respectively. One hundred and eighty catfish juvenile were reared in 18 aquarium. They were fed with various concentration of prebiotic mixture for 14 days. They were infected with 1 mL *A. hydrophila* at 1.2×10^5 cell/cc intramuscularly and observed for 10 days for their survival and immune response. Results showed that addition of red ginger and moringa leaf extract significantly improve the immunity of catfish. The addition of moringa leaf extract at 600 ppm was significantly improved survival rate, phagocytosis activities, leucocyte, lymphocytes count compared to other prebiotic mixtures. Thus, the usage of 600 ppm moringa leaf extract in the diet provided the best performance to catfish.

1. Introduction

Catfish (*Clarias gariepinus*) has been known as the most popular species to be culture and mostly produced in small scale and back yard system. However, this species has significantly increase fish production as well as protein intake. In 2017 the catfish production was 1.77 million tons and it was increased 131.7 % above the target which was 1.30 million tons [4]. This production potentially be increased if disease problems could be overcome.

Many attempt has been done by the farmer to avoid mass mortality due to bacterial disease. One them was the use of antibiotics. There were 67 antibiotics and 73% were using oxytetracycline [15]. The increase concern of the usage of antibiotic in aquaculture for food safety and human health has triggered interest on the use of organic compound from plant extract as immunostimulant, antibacterial and growth promotor [3,6]. Several organic plant compound has been proven to be affective as bacteriostatic such as temu kunci (*Kacipferia pandurata*) [10], red ginger [17], mangrove leaf extract [17]. Previous study demonstrated that ethanol and methanol tri herbal solvent extract from *Azadirachta indica*, *Ocimum sanctum* and *Curcuma longa* were able to stimulate innate immune mechanisms such



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as phagocytosis activity, respiratory burst activity, lysozyme activity and disease resistance in goldfish (*Carassius auratus*) against *Aeromonas hydrophila* [9]. Further research found that red ginger extract at the concentration of 600 ppm was able to retain survival rate of infected gourami to 56.67% and not significantly reduced the growth rate [17]. Whilst other researchers stated that the use of 150 ppm moringa leaf extract was able to manage survival of infected sangkuriang catfish with *A. hydrophila* at 80% and stimulates white blood cells $23,46 \pm 6,46\%$ [19].

The aims of this research was to observe the performance of the ethanol solvent mixture of red ginger and moringa leaf extract as prebiotic in the diet to the survival and immune response of catfish infected by *A. hydrophila*.

2. Material and methods

The research was conducted from February to June 2019 at Aquaculture laboratory, Faculty of Fisheries and Marine Science, Diponegoro University. The catfish fingerlings (*C. gariepinus*) used in this study were collected from Ambarawa hatchery with the body weight around 7 – 9 grams. The total fingerlings used were 180 animals and stocked in 18 aquariums. Thus each aquarium contained 10 fishes.

An experimental laboratory using a completely randomized design with 6 treatments and 3 replications were used in this study. The treatments were a mixture of ethanol solvent red ginger and moringa leaf extract at the concentration of (A) 500 ppm and 0 ppm, (B) 375 ppm and 150 ppm, (C) 250 ppm and 300 ppm, (D) 125 ppm and 450 ppm, (E) 0 ppm and 600 ppm, (F) 0 ppm and 0 ppm respectively. Extract of both red ginger and moringa leaf were prepared according to the method published by [18,19]. Experimental fish was reared and feed with various concentrations of red ginger and moringa leaf extract mixture for 10 days to stimulate the immune system. After that, experimental animals were infected by pathogenic bacteria *A. hydrophila* at concentration of 1.2×10^5 cell/cc through 0.1 mL intramuscular injection. Clinical behavior was observed every two days post-infection, while survival rate and growth were measured at the end of the research and the immunological response was observed every 3 days. Data were then analyzed using descriptive and analysis of variance. A further test was applied when there were any significant differences in the analysis of variance.

3. Results and discussion

The direct observation of the experimental fish prior and after being infected by *A. hydrophila* showed in table 1. It was shown that none of the experimental catfish were demonstrated abnormality behavior during 10 days of feeding with various concentrations of red ginger and moringa leaf extract mixture. All experimental fish were shown to stop feeding, aggressiveness and some were hanging below water surface at the first two days post infected by *A. hydrophila*. The appetite of catfish was decreased significantly at all treatments until two days post infection (day 14) and started recover at day 16 then the appetite recovers from day 18 to 20 except control treatment (F). Similar trend was also demonstrated in the aggressiveness and hanging/stand still below the water surface. Those abnormalities behavior such as decreased appetite, lethargy, hanging, swimming imbalance were also reported by [1,2,22]. The decrease of abnormality behavior after 6 days of infection indicated that experimental fish entered a stage of recovery. These results in line with study conducted by [6,16] that organic compound from plant contain antibacterial substances that could control fish disease.

Table 1. Abnormal behaviour of the catfish infected by *A. hydrophila* that previously fed with various concentration of mixture red ginger and moringa leaf extract

Day of obs	A			B			C			D			E			F		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
12	++	+	++	++	+	++	+	+	++	+	++	+	+	+	+	++	++	++

14	+	+	+	+	+	+	+	+	+	+	+	+	+	+	++	+	++	
16	-	-	+	+	+	+	+	+	+	+	-	+	+	-	-	++	+	++
18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	++	+	+
20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-

Note :

A. Red ginger and moringa leaf extract 500 ppm and 0 ppm, B. 375 ppm and 150 ppm, C. 250 ppm and 300 ppm, D. 125 ppm and 450 ppm, E. 0 ppm and 600 ppm, F. 0 ppm and 0 ppm

1. Feeding, 2. Aggressive, 3. Hanging

- None, + mild, ++ moderate

Leucocyte profile of catfish during the experiment is presented in Figure 1. From Figure 1, it is clear that addition of the mixture of red ginger and moringa leaf extract significantly stimulated leucocyte production ($4.01 - 7.3 \times 10^4$ cell/cc) compared to control (F, $1.85 - 4.25 \times 10^4$ cell/cc). Furthermore, a mixture of red ginger and moringa leaf extract at a concentration of 250 ppm and 300 ppm (C), 125 ppm and 450 ppm (D), and 0 ppm and 600 ppm (E) were significantly increased immune response against pathogen than control (F)

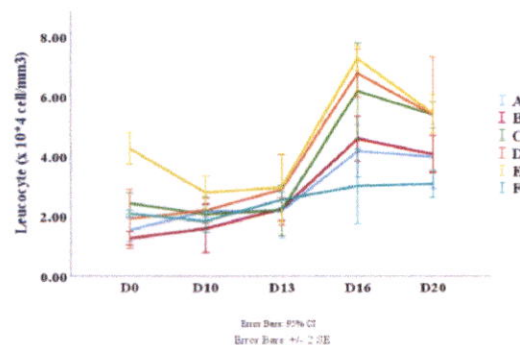


Figure 1. Leucocyte performance of catfish fed with mixture of red ginger and moringa leaf extract during the experiment.

Actually, the increase of leucocyte after infection is a natural response of any living organism. This was supported by [13] that the increased of leucocyte was a response of immune system against foreign body or pathogen that potentially spoiled host. Further it was estimated that leucocyte number for fish ranged from 20.000 – 150.000 cell/cc [14]. Thus, the number of leucocyte prior to treatment were just at the minimum level then increased significantly after infection. However, the leucocyte level was within normal range. Lymphocyte and phagocytic index of catfish (*C. gariepinus*) fed with mixture of red ginger and moringa leaf extract during the trial was presents in the Figure 2a and 2b.

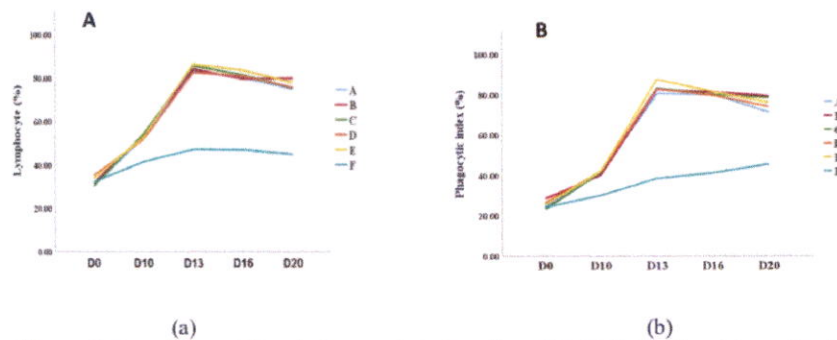


Figure 2. Lymphocyte (a) and phagocytic index of cat fish (b) fed with mixture of red ginger and moringa leaf extract during the experiment.

Lymphocyte is a cell that responsible for an immune response. Lymphocyte will be produced and mobilized when foreign body was detected in the fish organ. Figure 2 a demonstrated that lymphocyte was produced quite significantly at the day of *A. hydrophila* infection (day 10). Then stable at around 71.2 – 86.5%. This value far above experimental fish that not fed with a mixture of red ginger and moringa leaf extract (32.2 – 47.2%). Similarly, phagocytic activity is also an immune response of the present of foreign body, and this was activated when infection was detected. Phagocytic index of experimental catfish was higher throughout the treatments compared to control (F). The value is 23.2 – 87.5% for treatment A to E and as low as 24.2 – 45.5% for control (F).

Leucocyte, lymphocyte and phagocytic index were a good indicator for the immunological status of experimental catfish during curing period. These results were in line with the previous study, which found that fish treated with immunostimulants usually show enhanced phagocytic cell activities [3,17,20].

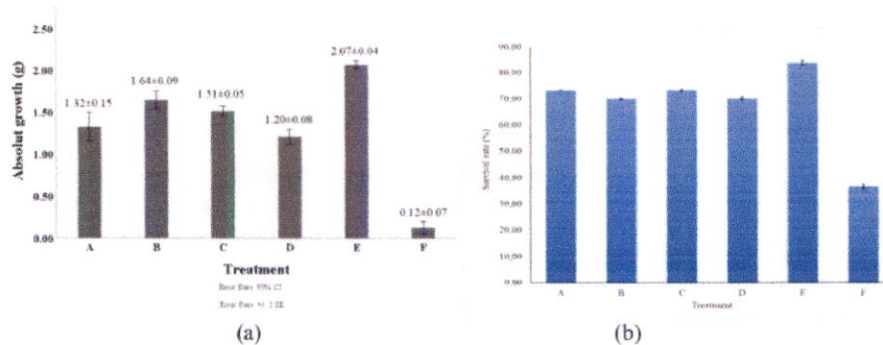


Figure 3. Growth (a) and survival rate (b) of cat fish (b) during experiment

Average growth performance as an effect of feeding treatment with diets containing various mixture concentrations of red ginger and moringa leaf extract for 10 days are presented in Figure 3a. The initial weight of tested catfish were similar ($P > 0.05$) ranging from 7 – 9 g/juvenile with an average of 7.80 ± 0.07 g. Experimental fish that fed with mixture of red ginger and moringa leaf extract seems to be able to cope with the *A. hydrophila* infection. These can be seen that at the end of the trial, the average growth rate was from 1.2 – 2.07 g/day whilst tested catfish with no treatment only grew 0.12 g/day. John

et al [12] demonstrated that four plant based immunostimulants (*Echinacea purpurea*, *Allium sativum*, *Nigella sativa* and *Origanum marjorana*) were able to improve weight gain than control.

The catfish feed with diets containing various mixture concentration of red ginger and moringa leaf extract showed a better survival rate compared to control (Figure 3b.). Analysis of variance showed that the addition of prebiotic into the diet for 10 days demonstrated a significant impact ($P < 0.05$) on the survival of catfish infected by *A. hydrophila*. Further test found that treatment E was the highest in survival rate (83,7%). Treatment E also resulted in the most consistent immune response namely leucocyte, lymphocyte and phagocytic index. These results indicated that addition of prebiotic moringa leaf extract in the diet for 10 days was able to stimulate catfish to produce immune system to combat *A. hydrophila*. The highest survival rate in treatment E was due to the content of active ingredients in this prebiotic, especially in the moringa leaf extract. The active ingredient such as : phenols, flavonoids, terpenoids, saponins and alkaloids in these extracts work to help the host dismantle antigens. Two bioactive compounds, namely saponins and flavonoids, have an important role against *A. hydrophila*. These bioactive compounds are denaturing proteins and damaging the bacterial cell membrane through inhibition of bacterial cell wall permeability, bacterial cell protein synthesis, bacterial cell nucleic acid synthesis, and bacterial cell metabolism [7]. Due to the ability of this prebiotic against bacteria and improving the immune response, the result found that adding this prebiotic extract moringa leaf at 600 ppm in the diet was able to improve the survival rate of juvenile catfish infected by *A. hydrophila* up to 83,7 %.

4. Conclusion

The addition of ethanol solvent extract of red ginger and moringa leaf extract in the diet significantly improve the immunological response of catfish to cope with bacterial *Aeromonas hydrophila* infection. The usage of ethanol solvent extract moringa leaf at 600 ppm was significantly improved survival rate, phagocytic index, leucocyte and lymphocytes count as well as growth rate compared to control.

Acknowledgment

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