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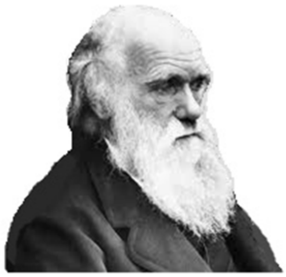

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Immune performances of sangkuriang catfish (*Clarias gariepinus*) with the addition of potassium diformate on feed

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ABSTRACT

This research aims to determine the effective dosage of potassium diformate (KDF) in feed to enhance sangkuriang catfish immune performances. The research was conducted on November - January 2019, at Aquaculture Laboratory Building No. 4 Faculty of Fisheries and Marine Sciences Universitas Padjadjaran. This study used an experimental method of Completely Randomized Design (CRD), with four treatments and three replications. The treatments are the addition of potassium diformate into feed with doses A (0%), B (0.5%), C (0.7%) and D (0.9%). Observed parameters are clinical symptoms, total leukocyte count, total erythrocyte count, specific growth rate, survival rate and water quality. The results showed that 0.5% KDF feed produced the highest survival rate of 85% and the highest specific growth rate of 3.59% after 40 days. Furthermore, due to treatment, total erythrocyte count increased from 2.09×10^6 cells/mm^3, to 2.10×10^6 cells/mm^3 (8.1%) and total leukocyte count increased from 7.1×10^4 cells/mm^3, to 10.7×10^4 cells/mm^3 (42.81%). The addition of 0.5% KDF in feed is effective in improving the immune performances of sangkuriang catfish challenged by *A. hydrophila*, with the highest survival rate of 97%. Moreover, recovery of total leukocyte count and total erythrocyte count was faster than other treatments as of day 7th, with a value 1.97×10^6 sel/mm^{3}and 12.60×10^4 sel/mm^3. It also resulted in the lowest gross clinical signs and the fastest recovering.

***Keywords*:** *Clarias gariepinus*, *Aeromonas hydrophila*, Potassium Diformate, Immune

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1. INTRODUCTION

Sangkuriang catfish is one type of freshwater fish that is much in demand by the people of Indonesia and is widely cultivated mainly on the island of Java. Sangkuriang catfish is one of the superior strains produced by researchers in the country. This catfish is the result of genetic catfish improvement carried out by the Center for Development of Freshwater Cultivation (BBPAT) Sukabumi by backcrossing the African catfish parent in Indonesia.

Constraints that are often faced in the business of catfish farming are susceptibility to disease, especially in seed size. One of the pathogenic diseases that often attacks catfish is Motile Aeromonas Septicaemia (MAS) caused by the bacterium *Aeromonas hydrophila* (Vivas *et al*., 2004).

Prevention is a disease control that is carried out before the fish catches the disease, this action is more efficient than treatment. One method of prevention carried out by farmers is by giving antibiotics. However, the uncontrolled use of antibiotics often has a negative impact on fish and the environment. The alternative to overcome this problem is by giving feed supplements. The organic acid is one of the feed additives used as immunostimulants which can inhibit pathogenic bacteria in the digestive tract (Berchieri, 2000). Organic acids that are often used are potassium diformate which can help the absorption process in the digestive tract.

Potassium diformate can be used as an alternative growth driver and health (Chowdhury *et al*., 2008). Potassium diformate can acidify the cytoplasm of gram negative bacteria, such as *Aeromonas hydrophila* and *Vibrio anguillarum* which ultimately results in cell death (Nermeen and Naela, 2015). The purpose of this research is to determine the effective dosage of potassium diformate in feed to improve the immune performance of sangkuriang catfish (*Clarias gariepinus*).

Taxonomic Hierarchy

Kingdom	Animalia – Animal, animaux, animals
Subkingdom	Bilateria
Infrakingdom	Deuterostomia
Phylum	Chordata – cordés, cordado, chordates
Subphylum	Vertebrata – vertebrado, vertébrés, vertebrates
Infraphylum	Gnathostomata
Superclass	Actinopterygii – ray-finned fishes, spiny rayed fishes, poisson épineux, poissons à nageoires rayonnées
Class	Teleostei
Superorder	Ostariophysi
Order	Siluriformes – silures, catfishes
Family	Clariidae Bonaparte, 1846 – airbreathing catfishes, labyrinth catfishes, poissons-chats à labyrinths, bagres laberintos
Genus	*Clarias* Scopoli, 1777 – walking catfishes
Species	*Clarias gariepinus* (Burchell, 1822)

2. MATERIALS AND METHODS

The material used in this research is sangkuriang catfish from ±7 cm Babakanjawa Majalengka Fish Seed Center, potasium diformate, Hi-Pro-Vite 781-1 commercial feed, and *Aeromonas hydrophila* bacterial isolates. This research uses experimental methods with Completely Randomized Design (CRD), consisting of four treatments and three replications. Each treatment uses 20 sangkuriang catfish. The treatment given is the addition of potassium diformate into feed with a dose that is treatment A (without the addition of KDF or Control), B (0.5% KDF), C (0.7% KDF), and D (0.9% KDF).

2. 1. Procedure

2. 1. 1. Preparation

Research preparation starts from the preparation stage of the container. Aquarium like fiber cleaned first soaked chlorine solution 10 ppm for 24 hours, then washed and dried. Then the aquarium is filled with 30 liters of water aerated for 24 hours. The next stage is the acclimatization of test fish to fiber tubs for 7 days. Then mixing feed with formatted potassium. Next prepared commercial feed and potassium diformate. The weighing of 100 grams of commercial feed was carried out for each treatment. Then weighed potassium diformate according to each treatment, as much as 0.5%, 0.7%, and 0.9% in 100 grams of feed. Then potassium diformate mixed with feed in a tray by stirring until evenly distributed. Feed that has been mixed with water is 10% of the total feed. The feed mixture is left at room temperature and dried for about 30 minutes.

2. 1. 2. Main Research

The main research starts from the maintenance phase of the test fish for 40 days by feeding according to the treatment three times a day, measuring the quality of water every 10 days, observing growth, retrieving red blood cells and white blood cells before the fish are treated, after being treated, and during the challenge test five times. Then the next step is a challenging test with *A. hydrophila* after 40 days of maintenance, bacterial infection is 0.1 mL per head and clinical symptoms are observed for 14 days after infection.

2. 2. Parameter Observation

The parameters observed were macroscopic clinical symptoms, total leukocyte count, total erythrocyte count, specific growth rate, survival rate, and water quality. Data on total leukocyte count, total erythrocyte count, specific growth rate, and survival rate were analyzed using F test and multiple distance test at 95% confidence level, while macroscopic clinical symptoms data and water quality were analyzed descriptively.

3. RESULT AND DISCUSION

3. 1. Macroscopic Clinical Symptoms

Observation of clinical symptoms on sangkuriang catfish seeds as test fish was carried out after the infection of *Aeromonas hydrophila* bacteria. Clinical symptoms observed were damage to fish bodies and changes in fish behavior. Observation results of damage to fish bodies can be seen in **Table 1**.

Table 1. Body Damage

Days to-	Potassium Diformate Dosage (gr)											
	A (Control)			B 0.5			C 0.7			D 0.9		
	Replications											
	1	2	3	1	2	3	1	2	3	1	2	3
1	ab	abc	abc	a	a	ab	ab	ab	a	a	ab	a
2	abc	abc	abc	abc	abc	ab	abc	abc	abc	abc	abcd	ac
3	abc	abcd	abc	abc	abc	ab	abc	abc	abcd	abc	abcd	ac
4	ab	abd	ab	abc	abc	b	abc	abc	abd	abc	abd	abcd
5	abc	b	ab	ab	ac	b	ab	abc	abc	abc	abd	abcd
6	abc	b	abc	b	ac	b	ac	abc	bc	abc	ab	abc
7	abc	bc	abc	b	c	b	ac	abc	bc	abc	abc	abc
8	bc	bc	bc	-	c	-	c	ab	c	ab	bc	abc
9	bc	bc	bc	-	-	-	c	b	c	ab	bc	abc
10	b	b	c	-	-	-	-	-	-	b	b	bc
11	-	-	-	-	-	-	-	-	-	b	b	bc
12	-	-	-	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-	-	-	-	-

Information: (a): Red stain (haemorrhagic), (b): Damaged fin, (c): Distended stomach (dropsy), (d): ulcer, (-): No clinical symptoms

Based on the results of observations of clinical symptoms in the test fish began to appear 1 hour after infection. Test fish in all treatments on the first day after infection with *A. hydrophila* bacteria showed signs of clinical symptoms of *A. hydrophila*. In treatment A (Control) the seeds experienced more severe clinical symptoms, such as the presence of red spots (**Figure 1b**), damage to the fins (Figure 1**c**), and the belly of the fish bulging (Figure 1**d**).

Clinical abnormalities, namely red spots, are a form of resistance of white blood cells to pathogenic bacteria and destroy them by phagocytosis.

(a) Healthy fish

(b) Haemorrhagic

(c) Damaged fin

(d) Dropsy

(e) Ulcer

Figure 1. Difference between Healthy Catfish and Affected by *A. hydrophila*

On day 2, the clinical symptoms that appear in the test fish at each treatment began to be evenly distributed. In treatment D (0.9%) clinical symptoms appear more severe. In treatment D (0.9%) the clinical symptoms appear to be more markedly marked by infection wounds in the area of the injection site (Figure 1**e**), the emergence of infected wounds in the former injection area because the area was the first to contact the *A. hydrophila* bacteria. On the 3rd and 4th day there were clinical symptoms that were getting worse in each treatment. The presence of ulcers were in treatment A (Control), C (0.7%) and D (0.9%). Then on the 5th to the 8th day of each treatment the condition of the fish gradually improved. Red spots on fish begin to disappear slowly. Furthermore, on day 9 to day 14, treatment A (Control), C (0.7%) and D (0.9%) also gradually improved and in treatment B (0.5%), as a whole did not, there is an indication that the fish is attacked by *A. hydrophila*. The rapid healing process is caused by the addition of formatted potassium that can increase the body's immunity and inhibit the attack of *A. hydrophila* bacteria.

Sangkuriang catfish seeds, infected with *A. hydrophila* bacteria in addition to physical changes, catfish also experience behavioral changes in the form of fish response to feed and surprises. A seed response to feed was observed for each feeding during the challenge test (**Table 2**). Observation of response to feed was carried out for 14 days to determine the response of fish to consume the feed given and to see the remaining feed that was not consumed.

Table 2. Response to feed

Potassium Diformate Dosage (gr)												
Days to-	A			B			C			D		
	(Control)			0.5			0.7			0.9		
	Replications											
	1	2	3	1	2	3	1	2	3	1	2	3
1	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	+	+	+	+	+	-	-	-	-
4	+	+	+	++	++	++	++	++	+	+	+	+
5	++	++	++	++	++	++	++	++	++	+	+	+
6	++	++	++	++	++	++	++	++	++	++	++	++
7	++	++	++	++	++	++	++	++	++	++	++	++
8	++	++	++	++	++	++	++	++	++	++	++	++
9	++	++	++	++	++	++	++	++	++	++	++	++

Days to-	Potassium Diformate Dosage (gr)											
	A			B			C			D		
	(Control)			0.5			0.7			0.9		
	Replications											
	1	2	3	1	2	3	1	2	3	1	2	3
10	++	++	++	++	++	++	++	++	++	++	++	++
11	++	++	++	++	++	++	++	++	++	++	++	++
12	++	++	++	++	++	++	++	++	++	++	++	++
13	++	++	++	++	++	++	++	++	++	++	++	++
14	++	++	++	++	++	++	++	++	++	++	++	++

Information: (-) No response
(+) Response to feed decreases
(++) Response to feed normal

Sangkuriang catfish seeds in the treatment A (Control) showed a slow response to the feed given. This is because the fish's immune system decreases due to the attack of *A. hydrophila*. Fish infected with *A. hydrophila* bacteria cause a decrease in appetite. In treatment B (0.5%), and treatment C (0.7%) it gave a faster response to feed.

This is thought to increase fish immunity because the active ingredients of KDF given during maintenance can help stimulate the work of the fish body's immune system or immunostimulants. This is consistent with Raa (2000) that immunostimulants work as a simultaneous approach to various pathogens by increasing the nonspecific immune response of fish.

Treatment D (0.9%) shows that the response to feed given is very slow. This is thought to be due to the injured gastrointestinal tract, because it can be seen from the clinical symptoms of fish caused by ulcers on the abdomen (Figure 1e). This is because more doses of potassium diformate added to the feed have not been able to increase fish immunity against the attack of *A. hydrophila* bacteria. *A. hydrophila* bacteria breed in the intestine, which causes bleeding in the intestine.

The same opinion is expressed that enteroxin compounds released by *A. hydrophila* attack the gastrointestinal tract in fish.

The change in behavior which was then observed was the response of sangkuriang catfish seeds to surprises. Observation of the response to shock or fish reflexes was carried out to determine the health condition of the fish for stimulation in the form of a shock in the motion of fish. This observation was also carried out for 14 days during the challenge test (**Table 3**).

Table 3. Response to shock

Days to-	Potassium Diformate Dosage (gr)											
	A			B			C			D		
	(Control)			0.5			0.7			0.9		
	Replications											
	1	2	3	1	2	3	1	2	3	1	2	3
1	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	+	+	+	+	+	+	-	-	-
4	+	-	+	++	++	++	++	+	++	+	-	+
5	++	++	++	++	++	++	++	++	++	++	++	++
6	++	++	++	++	++	++	++	++	++	++	++	++
7	++	++	++	++	++	++	++	++	++	++	++	++
8	++	++	++	++	++	++	++	++	++	++	++	++
9	++	++	++	++	++	++	++	++	++	++	++	++
10	++	++	++	++	++	++	++	++	++	++	++	++
11	++	++	++	++	++	++	++	++	++	++	++	++
12	++	++	++	++	++	++	++	++	++	++	++	++
13	++	++	++	++	++	++	++	++	++	++	++	++
14	++	++	++	++	++	++	++	++	++	++	++	++

Information : (-) No response
(+) Response to shock decreases
(++) Response to shock normal

Based on the observations on day 1 to day 2, there was no response to the shock given to all treatments. This is presumably because the test fish experienced an attack from *A. hydrophila*, so the test fish experienced a decrease in response to shock and stress.

In treatment A (Control) and treatment D (0.9%), from day 1 to day 3, there was no response to the surprises given. This shows that the fish is sick due to the attack of the *Aeromonas hydrophila* bacteria, which results in reduced appetite and damage to the body of the sangkuriang catfish seeds and thus lack of nutrient intake. Unlike treatment B (0.5%) and treatment C (0.7%), the third day of the test fish responded to the shock faster. This is in accordance with the results of observations of the response to feed (Table 3) which shows the fish in sick condition, but not as severe as compared to the Sangkuriang catfish treatment A (Control) and D treatment (9%).

In treatments A (Control) and treatment D (0.9%) experienced a response to very slow surprises. The response of fish begins to appear on the 4th day. On the 5th day until the end of the maintenance period the fish begins to show a response to surprises, this can be seen from the beats on the aquarium wall with the response given by the fish moving quickly or agile, whereas in treatment B (0.5%) from day 4 to the end of the test period the fish gave a normal response. This can be seen from the shock results are given by the fish moving quickly because the effect of KDF given effectively increases the body's immunity against the attack of *A. hydrophila*.

3. 2. Total White Blood Cells Count (Leukocyte)

Observation of white blood cell count was carried out to determine changes in white blood cells in sangkuriang catfish. The number of white blood cells is related to the fish's immune system. The results of observing white blood can be seen in **Figure 2**.

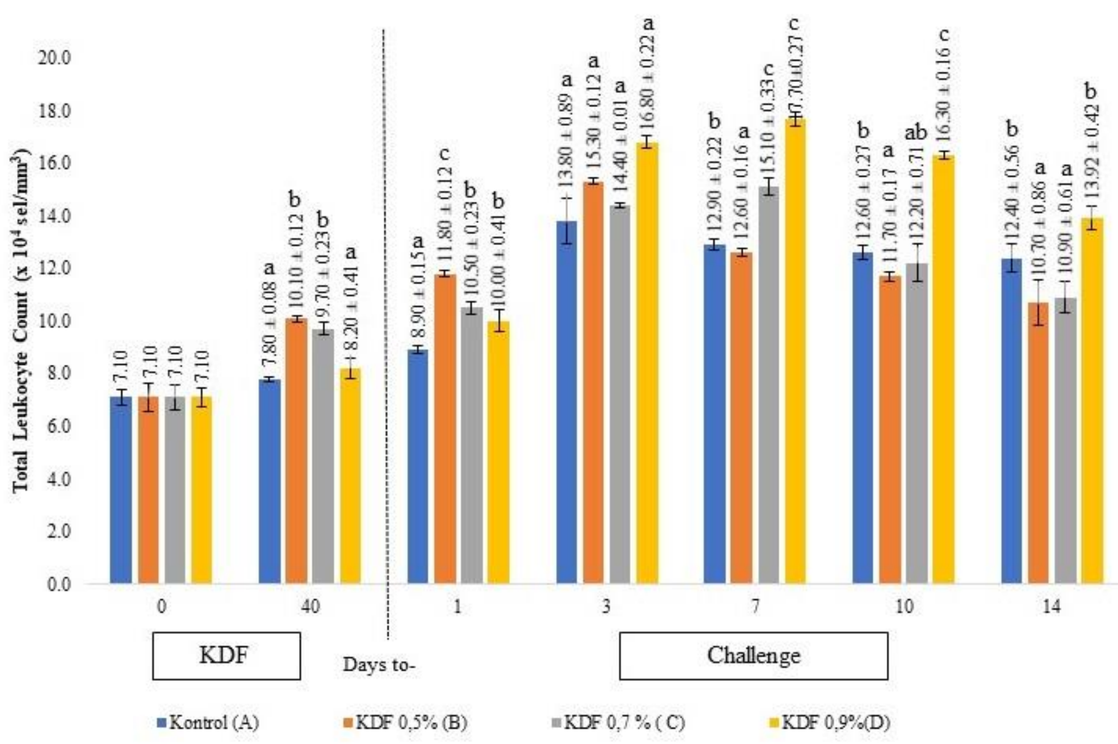

Figure 2. Total Leukocyte Count

Based on Figure 2, the number of total leukocyte count of sangkuriang catfish seeds after being treated with potassium diformate for 40 days which was added to the feed increased, but the number of white blood cells was still in the normal range (7.80×10^4 - 10.10×10^4 cells / mm^3). In accordance with Larger *et al*. (1977), the number of white blood cells in fish ranges from $2\text{-}15\times10^4$ cells / mm^3.

The addition of potassium diformate into feed, it affects increasing the number of white blood cells in sangkuriang catfish seeds. Test treatment B fish (0.5%) KDF produced the highest increase in white blood cells, which was 10.10×104 cells / mm^3. It is seen that KDF can increase the number of white blood cells. An increase in the number of white blood cells occurs because of the active substances contained in KDF. Active substances function as immunostimulants which can trigger fish immunity. According to Mas'ud (2013), one of the abilities of immunostimulants is to increase the resilience of non-specific fishes by increasing white blood cells which act as phagocyte cells.

Based on the analysis of variance, it showed that the addition of KDF to feed with different doses had a different effect on the number of white blood cells. Duncan's multiple distance test results showed that the Sangkuriang catfish seed treatment B (0.5%) KDF was significantly different from treatment A (Control) and treatment D (0.9%) but not significantly different from treatment C (0.7%).

At the time of the challenge test, the number of white blood cells generally increases. Test fish in treatment B (0.5%) had the lowest increase compared to other treatments. This is because the KDF in the feed given acts was as an immunostimulatory agent to improve the immune system of the sangkuriang catfish. So that, when there is an attack from the bacterium *A. hydrophila* the body's immune system can fight the bacteria. The active ingredient contained in KDF is known to increase the number of white blood cells in fish. The white blood cells formed are used to fight the attack of *A. hydrophila* bacteria. Then the number of white blood cells decreases as fast as normal. This is because white blood cells work optimally against the attack of *A. hydrophila* bacteria, so that fish become healthier.

Treatment D (0.9%) resulted in the highest increase in the number of white blood cells compared to other treatments. The high increase in the number of leukocytes in this treatment is because after infection with *A. hydrophila* the body's resistance or the body's immune system decreases, indicating that the fish is experiencing a bacterial attack, and it can be seen from the clinical symptoms that are worse than the other treatments (Table 1). As the opinion on white blood cells in fish have increased fourfold because fish are experiencing resistance to foreign substances (bacteria, viruses, parasites). In fighting bacterial attacks, the active ingredient in KDF is excessively less effective in increasing fish immunity.

3. 3. Total White Blood Cells Count (Erithrocyte)

Red blood cells are the most numerous blood cells. Red blood cells contain hemoglobin which allows red blood cells to carry oxygen from the lungs and deliver it to all body tissues. The results of the research on the number of red blood cells can be seen in **Figure 3**.

Treatment of B (0.5%) KDF showed a tendency for cells to have higher red blood cell counts of 2.26×10^6 cells / mm^3 compared to other treatments. In treatment A (Control) the lowest increase in the number of red blood cells was 2.12×10^6 cells / mm^3. Increasing the number of red blood cells due to the number of red blood cells is influenced by nutritional conditions, physical activity, and age (Dellman and Brown, 1989).

The results of variance analysis showed that the difference in the dose of KDF in the feed given did not give a significant difference to the number of red blood cells. But there is a tendency to increase the number of red blood cells higher than controls.

The number of red blood cells after being challenged with *A. hydrophila* bacteria decreased day 1 to day 3. This occurs due to *A. hydrophila* infection. State when *A. hydrophila* enters the body, the target of infection is blood vessels. *A. hydrophila* bacteria produce hemolysin enzymes that can lyse red blood cells, so that the number of red blood cells in blood vessels decreases. On the 7th day until the 14th day in general, the increase was almost normal, except for treatment D (0.9%). Treatment B (0.5%) experienced an increase in the number of blood red cells nearing normal speed. This is because the condition of the fish affected by *A. hydrophila* is improving because efforts in the body of the fish produce more red blood cells to replace the red blood cells that have been lysed.

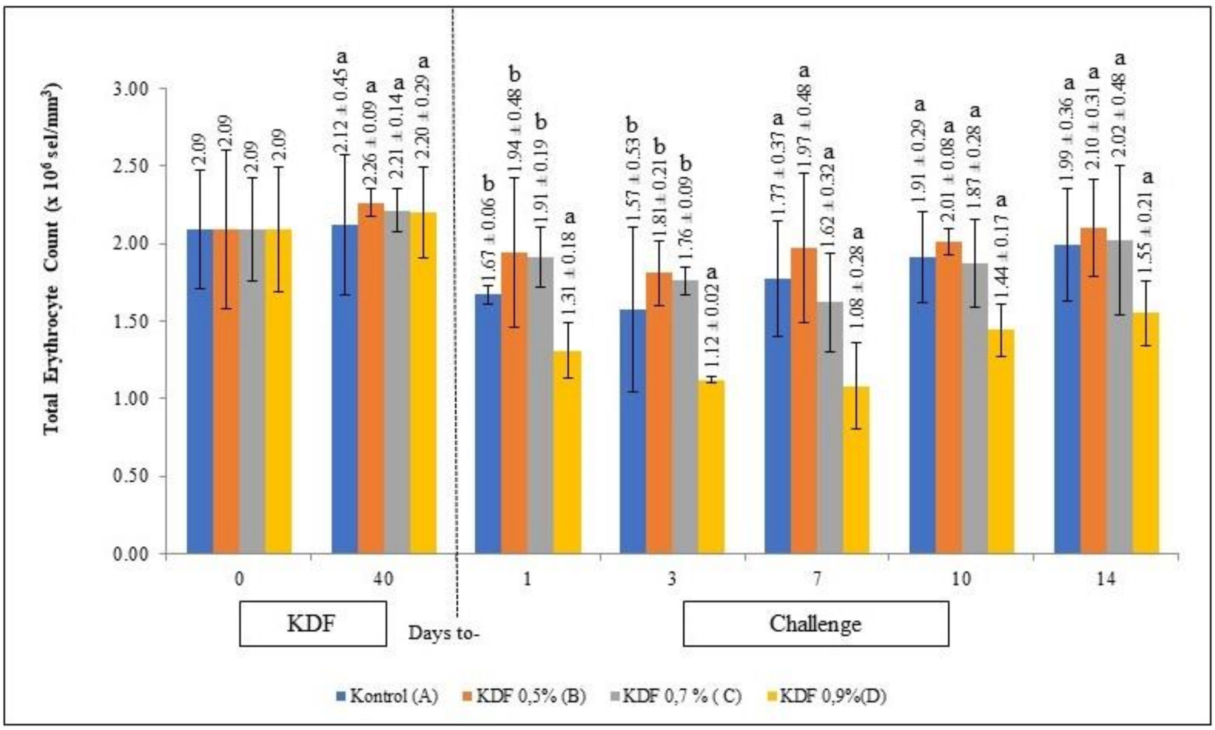

Figure 3. Total Erythrocyte Count

3. 4. Specific Growth Rate

The specific growth rate serves to calculate the percentage growth in weight of fish. The results of observations of specific growth rates on sangkuriang catfish seeds for 40 days during catfish rearing given the addition of KDF can be seen in **Figure 4**.

The specific growth rate of sangkuriang catfish seeds with the addition of KDF to the feed is higher than without the addition of KDF. Treatment of B (0.5%) KDF produces the highest specific growth rate of 3.59%. This shows that the absorption process in the digestive tract occurs optimally. Formatted potassium has an antimicrobial effect on gram negative bacteria by penetrating through the bacterial cell wall and then releasing protons. So that bacteria need energy to balance pH, resulting in depleting energy and then dying. KDF can improve intestinal health with a stronger antimicrobial effect on *E. coli* bacteria than lactic acid bacteria. KDF is an organic acid that can reduce the pH of the digestive tract, thus activating pepsin to increase digestibility of proteins. The low pH of the digestive tract can also reduce pathogenic bacteria and increase lactic acid bacteria, because lactic acid bacteria can grow at a relatively low pH and are more resistant to organic acids or salts than Gram negative bacteria Nermeen (2015).

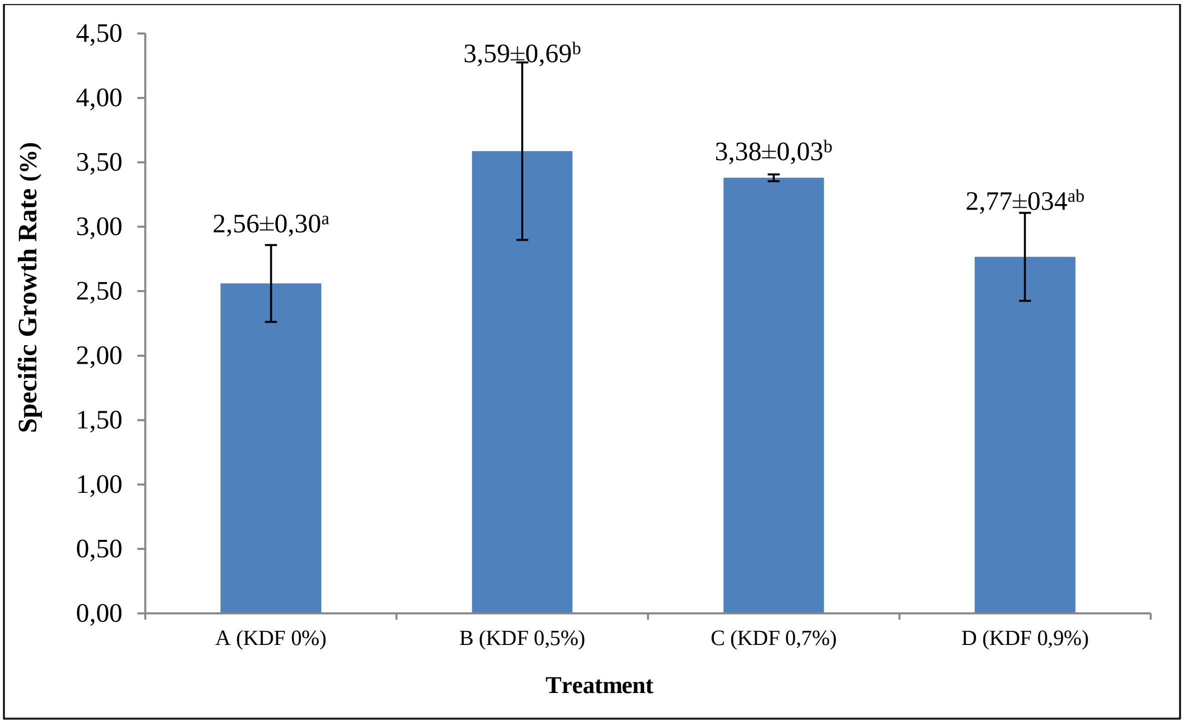

Figure 4. Specific Growth Rate

The D (0.9%) KDF treatment results in a lower specific growth rate of 2.77%. The higher dose of KDF given results in a lower specific growth rate. This is because the excessive doses of KDF are less effective in increasing growth. According to Arge *et al*. (2006), the use of organic acids exceeding the optimum level can damage the stomach mucosa and duodenum. Over-administration of KDF is also thought to interfere with the osmoregulation system, because fish use their energy to maintain their osmoregulation balance. Based on the analysis of variance showed that the addition of KDF to feed with different doses had a different effect on the specific growth rate of sangkuriang catfish. Duncan's multiple distance test results showed that sangkuriang catfish seeds were given treatment KDF B (0.5%) and treatment C (0.7%), were significantly different from the control treatment, and treatment D (0.9%) was not different from the control treatment, treatment B (0.5%) and treatment C (0.9%).

3. 5. Survival Rate

Survival rate observations were carried out during maintenance with the addition of potassium diformate (KDF) for 40 days and after the challenge test. Based on observations, each treatment produces different survival rate (**Figure 5**).

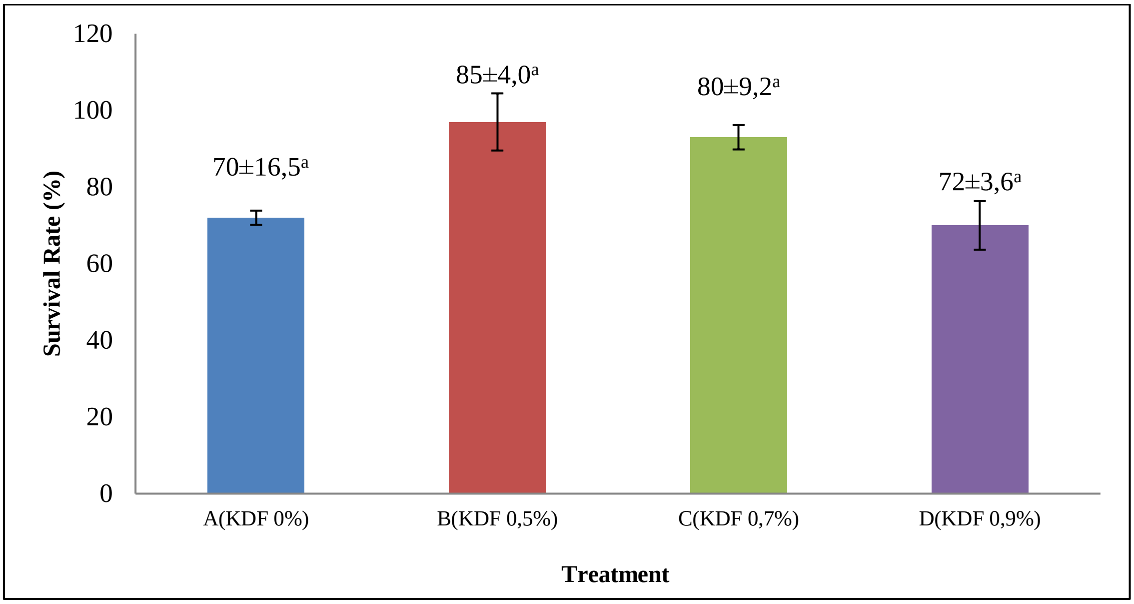

Figure 5. Survival Rate After Addition of KDF

Treatment of B (0.5%) KDF showed the highest trend of survival rate, while treatment of control A (0%) resulted in the lowest survival rate. This is because the addition of KDF contains active ingredients of organic acids which can optimize pH in the digestive tract and stop the growth of pathogenic bacteria. According to Partanen and Mroz (1999), organic acids and their salts have a beneficial effect on growth performance by increasing intestinal health through pH reduction, increasing bacterial growth which is beneficial while inhibiting the growth of pathogenic microbes. So that the health of fish will increase and can improve survival rate.

Based on the results of an analysis of variance one can see that the difference in the dose of KDF in the feed given did not provide a significant difference in the survival rate of the test fish. But there is a tendency for the survival rate of fish with the addition of KDF higher than the control. The observation survival rate of sangkuriang catfish seeds was also carried out after challenging tests with *A. hydrophila* for 14 days, as shown in **Figure 6**.

Addition of 0.5% KDF to feed resulted in the highest survival rate after the fish were tested challenged for 14 days at 97%, and the lowest survival rate at treatment D (0.9%) by 70%. The high survival rate is due to the active substance found in KDF works well to increase immunity. This is indicated by an increase in the number of white blood cells produced higher, the clinical symptoms that are caused to be lighter than other treatments, and the response to feed and surprise responds more quickly. These results are research with the addition of 0.5% KDF resulting in the significant white snapper survival rate of 98.3%. However, the higher the KDF dose given, the lower the survival rate.

This is due to the excess of active substances contained in the body of the fish so that it is less effective in increasing fish immunity to fight the attack of *A. hydrophila*. Seen from the test fish in treatment D (0.9%) resulted in the lowest increase in white blood cells, the most severe clinical symptoms, and the response to feed and shock was very slow. Organic acids added at excessive levels can have a negative effect on the digestion. Excessive use of organic acids can damage the stomach mucosa and duodenum.

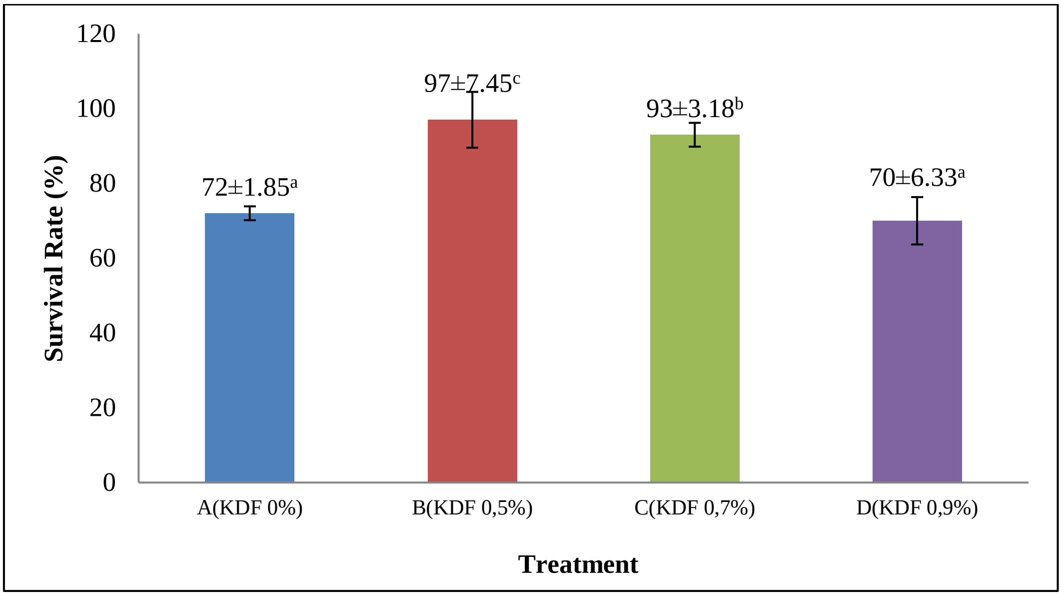

Figure 6. Survival rate Sangkuriang Catfish Seeds After Challenge

Based on the analysis of variance one can see that the addition of KDF to feed with different doses had a different effect on the survival rates of sangkuriang catfish which were challenged with *A. hydrophila* bacteria. Duncan's multiple distance test results showed that sangkuriang catfish seeds that were given KDF 0.5% were significantly different from other treatments, and the treatment (control) was not significantly different from the treatment of 0.9% KDF.

3. 6. Water Quality

Observation of water quality is used as a supporting, or supporting parameter during the study. The results of observing water quality during the study can be seen in **Table 4**.

Table 4. Water Quality Observation Results

Treatment	Water Quality Parameters		
	Temperature (ºC)	pH	DO ($mg \cdot L^{-1}$)
A (Control)	25.7-29.8	6.56-7.91	4.1-5.7
B (0.5%)	25.7-29.5	6.53-7.52	4.4-6.2
C (0.7%)	25.5-29.4	6.62-7.82	4-5.7
D (0.9%)	27.5-29.8	6.66-7.73	4-5.8
Optimal SNI (2002)	25-30	6.5-8.5	≥4

Based on the results of measurements of water quality during the study, obtained in the optimal range and meet the optimum standards are in accordance with the Indonesian National Standard (SNI) for the maintenance of sangkuriang catfish. Potassium diformate did not affect water quality because it was seen from the control treatment with potassium diformate treatment, the results were not much different.

4. CONCLUSIONS

The conclusion of this research is that 0.5% KDF feed produced the highest survival rate of 85% and the highest specific growth rate of 3.59% after 40 days. Total erythrocyte count was increased from 2.09×10^6 cells/mm^3 to 2.10×10^6 cells/mm^3 (8.1%), and total leukocyte count increased from 7.1×10^4 cells/mm^3 to 10.7×10^4 cells/mm^3 (42.81%). The addition of 0.5% KDF in feed is effective to improve the immune performances of sangkuriang catfish challenged by *A. hydrophila* with the highest survival rate of 97%. Recovery of total leukocyte count and total erythrocyte count took place faster than other treatments, on the day 7th with a value 1.97×10^6 sel/mm^3 and 12.60×10^4 sel/mm^3 and also showed the lowest gross clinical signs and the fastest recovering.

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