Original Research Article

The Use Of Pottasium Diformate In Feed to Improve Immunity Performance Of Common carp (*Cyprinus carpio*, L)

ABSTRACT

This research aims to determine the optimum potassium diformate (KDF) dosage which was added to commercial feed to increase the immune performance of common carp fingerlings (*Cyprinus carpio* L). This study was conducted from October – December, 2021 at the Laboratory of Aquaculture and Molecular Biotechnology Laboratory of the Faculty of Fisheries and Marine Sciences, Universitas Padjadjaran. The method used in this research was experimental in a Completely Randomized Design (CRD) with 5 treatments and 3 replications. The treatments are A (without KDF as control), B (0.2% KDF), C (0.3% KDF), D (0.4% KDF) and E (0.5% KDF). The observed parameters are total leukocyte count (white blood cells), total erythrocyte count (red blood cells), and Macroscopic Clinical Symptoms. Observations were made after 35 days of KDF and post-test challenge by *Aeromonas hydrophila* for 14 days. Total leukocyte count and total erythrocyte count were analyzed using the F test and Duncan's advanced test at a 95% confidence level, while the gross clinical sign was analyzed descriptively. The results showed that the KDF 0.3% was the most effective dose to increase the immune performance of common carp, the result showed that the number of white blood cells and red blood cells had the highest increase of 22,95% and 20,55% . In addition, the process of recovering is faster than other treatments it can be shown by the number of total leukocyte count and total erythrocyte count, with a value of 8,38 × 10^4 cells/mm^3, and 1,46 × 10^6 cells/mm^3 and symptoms healing time of Common carp.

Keywords : *Aeromonas hydrophila*, *Cyprinus carpio*, Potassium Diformate, Immune system

1. INTRODUCTION

Common carp is a freshwater fishery favored by the community [1]. The growth of carp is relatively fast but in a less efficient aquaculture system, it can result in unstable air quality which poses various threats to the life and health of fish. One of the diseases that often attack fish is red spot disease caused by *Aeromonas hydrophila* bacteria or commonly called *Motile Aeromonas Septicemia*. This disease often attacks freshwater fish, causing high mortality outbreaks [2]. Fish disease caused by the bacterium *A. hydrophila* became known in Indonesia around 1980, which caused disease in carp in West Java, resulting in 125 tons of deaths [3].

One of the alternative preventions carried out by farmers from pathogen attacks is by increasing the immune system in carp by giving feed additives as immunostimulants. Immunostimulants play a role in activating non-specific defense mechanisms, and specific immune responses [4]. The addition of organic acids to feed is one strategy that can be used to improve the health of cultured fish. Nutrient organic acids can promote growth and control pathogenic bacteria. The active chemicals contained can provide an antimicrobial effect against gram-negative bacteria so that it can increase the body's resistance [5]

The organic acid that is starting to be widely used is potassium formate (KDF). Potassium formate can improve feed efficiency, growth performance, kill pathogenic bacteria, and can increase survival. Potassium formate can lower the pH in the intestine so that it can reduce the activity of pathogenic bacteria. The organic acids contained can also maintain the balance of the bacterial population that is resistant to acidic conditions such as lactic acid bacteria which function as probiotics for immunostimulants. Beneficial bacteria in the digestive tract can improve intestinal health so that the fish's body condition will be stronger [6]

2. MATERIAL AND METHODS

Common carp fingerlings size 3-5 cm came from the Cibiru Fish Seed Center (BBI) in Bandung, West Java. 15 aquariums with a size of 40 cm × 25 cm × 28 cm were used as maintenance containers. Goldfish seeds were kept in an aquarium with a density of 10 fish/aquarium. The test fish were fed potassium-formatted feed for 35 days. The amount of feed given as much as 3% of body weight, namely at 08.00 West Inonesian time and at 16.00 West indonesian time. The method used is experimental using Completely Randomized Design (CRD) with 5 treatments and 3 replications, namely:
Treatment A :Control (Without addition of potassium formate), Treatment B : Addition of 0.2% formate potassium, Treatment C : Addition of 0.3% formate potassium ,Treatment D : Addition of 0.4% formate potassium, and Treatment E: Addition of 0.5% formate potassium

2.1 Mixing of potassium diformate with feed

The feed used in this commercial feed in the form of floating pellets with a protein content of 35%. The feed was weighed as much as 100 grams and mixed with potassium diformate according to the treatment, namely 0.2%, 0.3%, 0.4%, and 0.5%. Potassium format was mized into the feed then stirred and mixed with 10% water and aerated

2.2 Culturing Of aeromonas hydrophila bacteria isolates

Aeromonas hydrophila isolate was inoculated on Tryptic Soy Agar media and then incubated at 30^{0}C for 24 hours. Bacteria were harvested using an ose needle and dissolved in Tryptic Soy Broth media and then incubated using a shaker incubator at 37oC for 24 hours. The bacterial culture was inserted as much as 2 ml into the cuvette to calculate its density using a spectrophotometer with a wavelength of 540 nm to obtain a density of 10^8 CFU/ml.

2.3 Challenge test

Cammon carp fingerlings were challenged *using A. hydrophila* bacteria by intraperitoneal injection (injection into the body cavity) of 0.1 ml/individual with a density of 10^8 CFU/ml. After being challenged using the bacteria *A. hydrophila* observed for 14 days.

2.4 Observation of blood cells

Blood cells were observed six times, namely before treatment, after administration of potassium formatted, and after day 3, day 7, day 10, and day 14 of the challenge. Protecting white cells using Truck fluid and red blood cells using Hayem fluid. Blood fluid was dripped on a hemocytometer to be observed under a microscope.

2.5 Data analysis

Data on white blood cell count and red blood cell count were analyzed using analysis of variance F ANOVA test and Duncan follow-up test with 95% confidence level, while clinical symptoms were analyzed descriptively.

3. Results and discussion

3.1 White blood cells (leukocytes)

Leukocytes are components of blood cells that function as non-specific defenses that play a role in the fish's immune system. Changes in the number of leukocytes can be used as an indicator of disease in fish because the body will produce more leukocytes when foreign objects enter the body [7]. The following is the average number of leukocytes in common carp during the study (Figure 1).

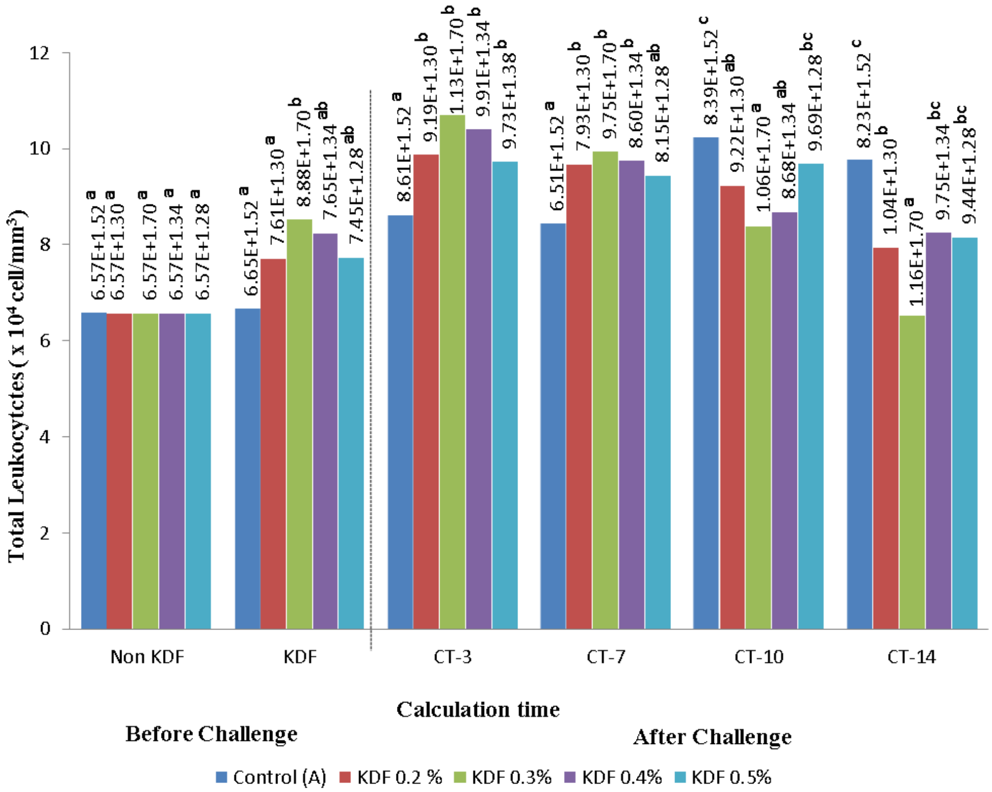

Figure 1. White blood cell (Leukocytes) count

Description:Nonn-KDF: before treatment KDF, KDF: KDF 35 days, CT-3: Third day after challenge, CT-7: Seventh after challenge, CT-10: Tenth days after challenge, CT-14: Fourteenth day after challenge

The graph (Figure 1) shows the average number of leukocytes after adding potassium diformate to the feed with different doses, white blood cells increased by 7.72 – 8.52 × 10^4 cells/mm3, the increase in the number of leukocytes occurred because KDF can increase cells White blood. Treatment C (0.3% KDF) experienced the highest increase in the number of leukocytes by 22.95% with a value of 8.52 × 10^4 cells/mm^3. The lowest was shown in Treatment A Enhancement (Without KDF) of 1.25% with a value of 6.65 × 10^4 cells/mm^3. This shows that the addition of KDF to feed at a dose of 0.3% can induce the immune system in fish.

The white blood cell count on the 3rd day after the away test showed an increase in the number of leukocytes between treatments. The highest increase occurred in treatment A (without KDF) of 22.70% with a value of 8.06 × 10^4 cells/mm^3. The highest changes in leukocyte values indicated that the fish had started to develop diseases caused by *A. hydrophila* bacteria. Changes in the number of leukocytes are more due to infection as a defense. These leukocyte cells work as cells that phagocytize bacteria so that they do not grow in the body may be this causes leukocyte cells to increase [8]. Fish that are infected will produce more leukocytes to maintain their immunity. The number of leukocytes with the lowest increase was on treatment C (0.3% KDF) with a value of 10.6 × 10^4 cells/mm3 of 20.21%. This shows that with the addition of 0.3% KDF the fish still have a good immune system. Changes in the number of leukocytes can be observed after 7 days after infection. This is because fish send more leukocytes due to infection as a defense. These leukocyte cells work as cells that phagocytize bacteria so that they do not develop in the host's body this causes leukocyte cells to increase [9]

The number of leukocytes on the day 14 (CT-14) of all treatments decreased. The decrease in the number of leukocytes in the Majalaya carp is the initial phase of healing in each treatment. The lowest decrease in treatment A (without KDF) was 4.9% with a value of 9.75 × 10^4 cells/mm^3 and the highest decrease in treatment C (0.3% KDF) was 28.8% with a value of 6.51 × 10^4 cells /mm^3.

3.2 Red Blood Cells (Erythrocytes)

Red blood cells function in binding oxygen which will then be used in the catabolism process that produces energy. Graph of the average number of red blood cells (Figure 2).

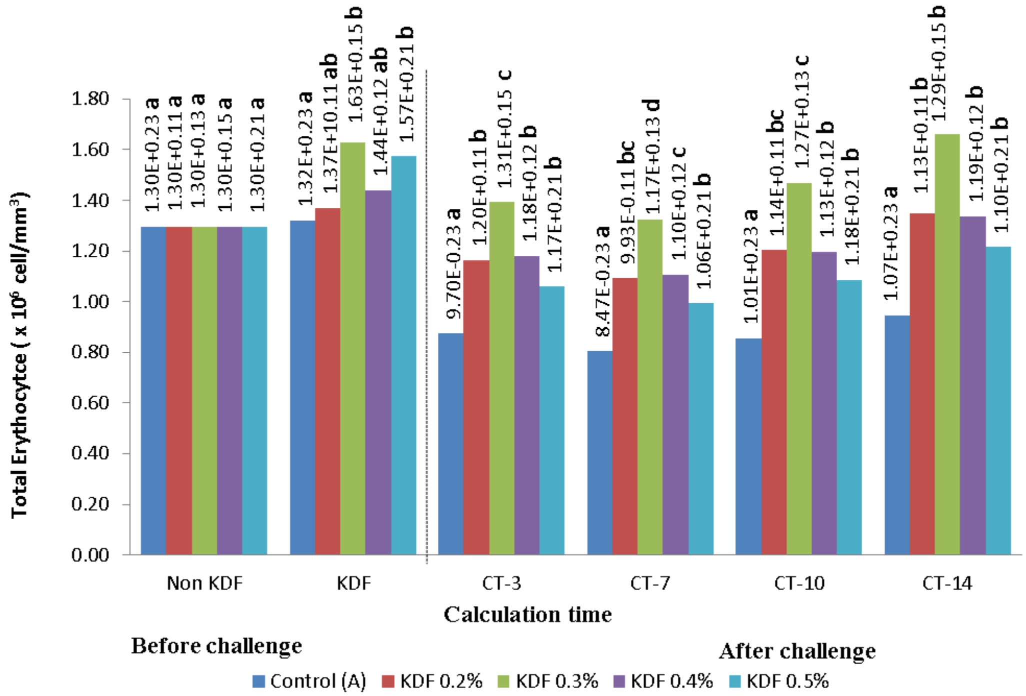

Fig. 2. Total Erythrocytes count

Description:Nonn-KDF: before treatment KDF, KDF: KDF 35 days, CT-3: Third day after challenge, CT-7: Seventh after challenge, CT-10: Tenth days after challenge, CT-14: Fourteenth day after challenge

The value of erythrocytes after administration of KDF for 35 days in treatment C (0.3% KDF) experienced the highest increase of 20.55% with a value of 1.63 × 10^6 cells/mm3. Treatment A (without KDF) experienced the lowest increase of 1.89% with a value of 1.32 × 10^6 cells/mm3.

The number of erythrocyte values after the day 3 away test decreased in each treatment. This happens because the fish lose red blood cells due to the presence of *A. hydrophila* bacteria in the fish's body. Red blood cells are hemoglobin which works as a carrier of oxygen throughout the body. A low number of red blood cells will cause fish to be unable to take in sufficient oxygen so that they will experience a lack of oxygen [10]. Low blood cell levels indicate anemia, while high red blood cells indicate the fish is in a state of stress [11]. Treatment A (without KDF) experienced the highest decrease in erythrocyte value, namely 50.6% with an average number of erythrocytes 0.88 × 10^6 cells/mm3, while the lowest percentage decrease was in treatment C (0.3% KDF) of 17% with an average value erythrocyte mean 1.39 × 10^6 cells/mm3. This is due to the presence of bacteria that may produce exotoxins and endotoxins that cause a decrease in red blood cells. The number of erythrocytes indicates an attempt at homeostasis in the infected fish body.

The number of erythrocytes on day 10 and day 14 after the challenge test was carried out in all treatments increased again. This happens because of the fish's recovery period to produce cells that have been infected. The highest number of cells was the treatment of increasing C (KDF 0.3%) on the 10th day around 9.77% and 11.65% on day 14 after the challenge test.

3.3 Macroscopic Clinical Symptoms

Observations of clinical signs in the fry of common carp were observed after a challenge test using *A. hydrophila* bacteria. Symptoms observed included morphological damage, response to feed, and fish response to shock

Table 1. Body Surface Damage for 14 days

Treatment	Test	Day to-													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
A	1	ab	abc	abcd	abcd	abcd	abc	bc	ac	acd	ac	ad	d	d	c
	2	ab	abcd	abcd	abcd	abc	abc	cd	c	acd	ac	d	d	d	cd
	3	ab	abc	abcd	abcd	abcd	abcd	bc	bc	acd	ac	d	d	d	c
B	1	ab	abc	abcd	acd	abcd	ab	cd	bc	ac	ac	d	d	d	cd
	2	ab	abc	abcd	abcd	bc	bc	ac	ac	cd	ac	d	d	d	d
	3	ab	abc	abcd	abcd	bc	ab	bc	ac	ac	ac	d	-	-	-

C	1	ab	abc	ab	abc	abc	abc	bc	c	cd	cd	d	d	d	d
	2	ab	abc	ac	bc	bc	bc	c	c	c	ac	d	d	-	-
	3	ab	abc	abc	ab	ab	ab	cd	cd	cd	d	d	-	-	-
D	1	ab	abc	abcd	abcd	abc	abc	d	c	cd	d	d	d	d	d
	2	ab	abc	abcd	acd	bc	bc	bc	cd	cd	ac	d	-	-	-
	3	ab	abc	abcd	abc	bc	bc	bc	bc	c	ac	d	d	d	-
E	1	ab	abc	abcd	abc	bc	abc	bc	bc	cd	cd	d	d	d	-
	2	ab	abc	abcd	abc	abc	ab	bc	bc	cd	ac	ad	d	d	d
	3	ab	abc	abcd	abc	ab	abc	bc	bc	c	cd	bd	d	d	d

Description: (a) Scales peeling (b) bleeding (haemorrhage) (c) distended abdomen (dropsy) (d) ulcers (ulcers)

Symptoms that appeared in each treatment were uneven (Table 1) because the test fish had different immune systems. Body surface damage and recovery period for each fish is different because each individual has a different resistance which is determined by age, sex, nutritional status, and stress [12]. Treatment C (0.3% KDF) showed better morphological resistance and a faster recovery period. Treatment A had more morphological damage than other treatments because there was no addition of KDF in the feed so the fish only got a low immunostimulant effect for their body resistance. *A. hydrophila* is a bacterium that grows in the blood vessels, causing bleeding and swelling symptoms such as ulcers and ulcers.

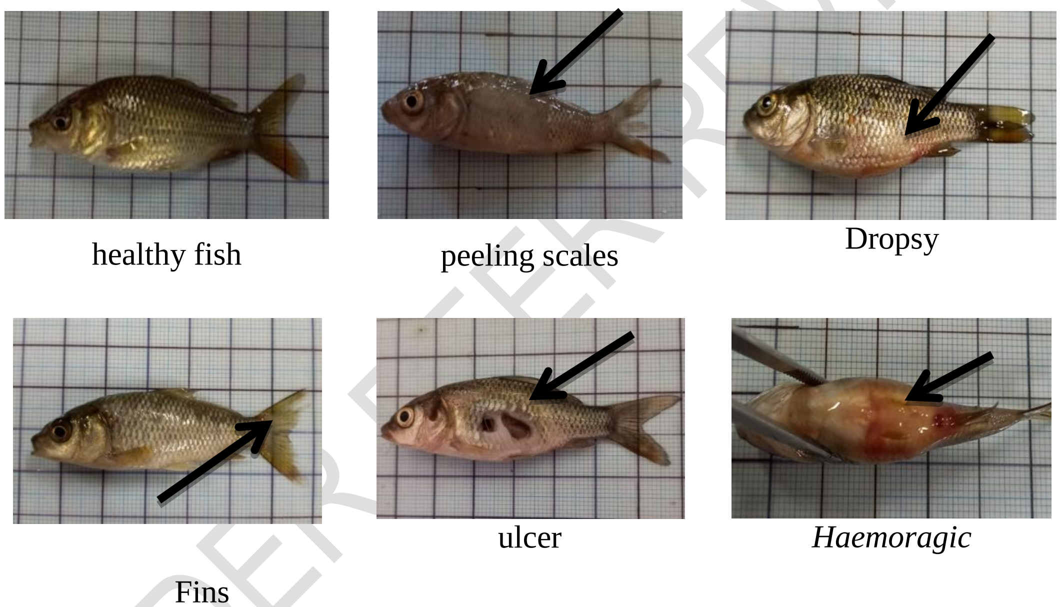

Fig 3: Different symptoms in fishes

Fish that are seriously injured continue to cause death. A. hydrophila is a bacterium that grows in the blood vessels, causing bleeding and swelling symptoms such as ulcers and ulcers. Infected fish will experience anemia and necrosis or ulcers on infected organs caused by the hemolysin enzyme in *A. hydrophila* bacteria lysing red blood cells and white blood cells [13]. *A. hydrophila* bacteria are bacteria that grow in blood vessels so that the symptoms that appear are related to bleeding and swelling.

Treatment E presents KDF with a dose of 0.5% is not effective because the salt contained in KDF can reduce palatability caused by acidifier so that the ability to release H+ is low, so it will inhibit the work of enzymes in the digestive tract [14]

Table 2. Response to Feed

Treatment	Test	Day to-													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
A	1	++	+	+	+	+	+	++	+	++	++	++	++	++	+++
	2	+	+	+	+	+	+	+	+	++	++	++	++	++	+++
	3	+	+	++	+	+	++	++	++	+++	++	++	+++	+++	+++
B	1	+	+	+	+	++	++	++	++	+++	+++	+++	+++	+++	+++
	2	++	+	+	+	+	++	++	+	+++	+++	+++	+++	+++	+++

	3	+	+	++	+	+	+	++	+	++	+++	+++	+++	+++	+++
C	1	++	+	+	++	++	++	++	++	+++	+++	+++	+++	+++	+++
	2	+	+	+	+++	++	++	+++	+++	++	+++	+++	+++	+++	+++
	3	++	+	++	++	++	++	+++	+++	+++	+++	+++	+++	+++	+++
D	1	++	++	++	+	++	++	++	++	+++	+++	++	+++	+++	+++
	2	++	++	++	++	++	++	+++	++	++	+++	+++	+++	+++	+++
	3	+	++	+	+	+	+	+++	+	++	++	+++	+++	+++	+++
E	1	+	+	+	+	+	+	++	+	++	+++	+++	+++	+++	++
	2	+	+	+	+	+	++	++	++	++	+++	+++	+++	+++	+++
	3	+	+	+	++	+	+	++	+	++	+++	+++	+++	+++	+++

Description : (+) Response to low feed (++) Response to moderate feed (+++) Response to normal feed

Observations on the response to feeding that had been injected using A. hydrophila bacteria showed a decrease in each treatment (Table 2). Disrupted fish metabolism causes a decrease in feed response. This decrease in feed response is caused by metabolic disorders so that internal organ abnormalities will occur in the form of swelling or inflammation of the liver, kidneys, and bile after injection [15].

Treatments C (0.3% KDF) and D showed the best feed response compared to treatments A (without KDF), B (0.2% KDF), and E (0.5% KDF) which had a lower response to feed. Treatment A had a lower feed response because of the large amount of leftover feed and decreased appetite response. The decreased feed response was caused by infection with *A. hydrophila* bacteria. Low red blood cells will cause the metabolic rate to decrease and the energy produced will also below. This will cause the fish to become weak and have no appetite look at the bottom and hang below the surface of the water.

On the 7th day after injection, the response to feed began to improve for treatments C and D, while for treatments A, B, and E on day 9. The addition of KDF to feed gave a better response to feed than without KDF, this was due to the active ingredient contained in KDF helping in boosting the fish's immune system so that the feed response will return to normal.

Table 3. Response to Surprise

Treatment	Test	Day to-													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
A	1	+	+	+	+	+	+	+	+	+	+	+ +	+ +	+ +	+ +
	2	+	+	+	+	+	+	+	+ +	+ +	+ +	+ +	+ +	+ +	+ +
	3	+	+	+	+	+	+	+	+ +	+ +	+ +	+ +	+ +	+ +	+ +
B	1	+	+	+	+	+	+	+	+ +	+ +	+ +	+ +	+ +	+ +	+ +
	2	+	+	+	+	+ +	+	+	+ +	+ +	+ +	+ +	+ +	+ +	+ +
	3	+	+	+	+	+	+	+	+ +	+ +	+ +	+ +	+ +	+ +	+ +
C	1	+	+	+	+	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
	2	+	+	+	+	+	+	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
	3	+	+	+	+	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
D	1	+	+	+	+	+	+	+	+ +	+ +	+ +	+ +	+ +	+ +	+ +
	2	+	+	+	+	+	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
	3	+	+	+	+	+	+	+	+ +	+ +	+ +	+ +	+ +	+ +	+ +
E	1	+	+	+	+	+	+	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
	2	+	+	+	+	+ +	+	+	+ +	+ +	+ +	+ +	+ +	+ +	+ +
	3	+	+	+	+	+ +	+	+	+ +	+ +	+ +	+ +	+ +	+ +	+ +

Description : (+) Response to shock is low (++) Response to shock

The fish response test to shock was carried out by knocking on the aquarium wall for each treatment. The results of post-challenge observations showed various responses to each treatment (Table 3). Response to shock on day 1 was low for all treatments. Fish infected with A. hydrophila bacteria will experience a decrease in a swimming motion and tend to swim on the surface of the water [16]. This is due to damage to the fins so that the swimming movement is not stable. Goldfish fry for treatment C (0.3% KDF) response to shock was normal on the 7th day and treatment A (without KDF) started to normalize to shock on the 11th day. Stated that A. hydrophila infection caused stress in fish, swimming around aeration, and in general, the fish swam sideways due to reduced body balance [17].

4. CONCLUSION

The conclusion that can be drawn from this research is that the optimum dosage of potassium diformate addition to feed is 0,3% dose to increase the immune system of the Common carp (*Cyprinus carpio L*) as seen from white blood cells (leukocytes), red blood cells (erythrocytes), clinical symptoms after A challenge test was conducted using *A. hydrophila* bacteria

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