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The Effect of Potassium Diformate as Feed Additive on Immune Performances of Nilem (*Osteochilus hasselti* Valenciennes, 1842) Under Infection of *Aeromonas hydrophila*

Ibnu Bangkit Bioshina Suryadi[1*], Desty Nurul Ulfa[1], Ayi Yustiati[1], Rosidah[1]

[1]*Department of Fisheries, Faculty of Fisheries and Marine Sciences, Universitas Padjadjaran, Jl. Raya Bandung Sumedang KM.21, Sumedang 45363, Indonesia*

[*]*Corresponding author: ibnu.bangkit@unpad.ac.id*

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ABSTRACT

This research aimed to determine the most effective dosage of potassium diformate additives in feed to increase the immune performance of nilem carp (*Osteochilus hasselti*). This research used Completely Randomized Design (CRD) experimental method with 4 treatments and 4 replications. The treatment used was the addition of potassium diformate on feed (0 %, 0.1 %, 0.3 % and 0.5 %). After 28 days of treatment, nilem fingerlings were challenged intraperitoneally with *Aeromonas hydrophila* bacteria with a density of 10^8 cfu ml^{-1} and then observed for another 14 days. Observed parameters were the number of leukocytes and erythrocytes, survival rate, clinical symptoms and water quality. The results showed that leukocytes and erythrocytes numbers were significantly different in each treatment. The leukocytes and erythrocytes numbers of nilem fingerlings after 28 days of potassium diformate treatments are higher than control. After challenged test, the numbers of leukocytes were increased until Day-10th due to the infection and decreasing to normal conditions on Day-14th. The numbers of erythrocytes were decreased until Day-10th due to hemolytic activity of *A. hydrophila* and increasing to normal conditions on Day-14th. Clinical symptoms after challenged test were relatively similar such as hemorrhagic, ulceration, exopthalmia, dropsy and abnormal swimming movements. It can be concluded that 0.3 % of potassium diformate addition has the highest survival rate after challenged test compared to other treatments (81.67 %), has the highest number of leukocytes and erythrocytes during infection, and recover faster than other treatments.

Keywords: *Aeromonas hydrophila, Erythrocyte, Immunostimulant, Leukocytes, Survival rate*

ABSTRAK

Penelitian ini bertujuan untuk menentukan dosis penambahan kalium diformat yang paling efektif dalam pakan untuk meningkatkan kinerja kekebalan ikan nilem (Osteochilus hasselti). Penelitian ini menggunakan metode eksperimen Rancangan Acak Lengkap (RAL) dengan 4 perlakuan dan 4 ulangan. Perlakuan yang digunakan adalah penambahan kalium diformat pada pakan (0%, 0,1%, 0,3% dan 0,5%). Setelah 28 hari pemberian pakan, benih nilem diuji tantang secara intraperitoneal dengan bakteri *Aeromonas hydrophila* dengan kepadatan 10^8 cfu ml-1 dan kemudian diamati selama 14 hari. Parameter yang diamati adalah jumlah leukosit dan eritrosit, tingkat kelangsungan hidup, gejala klinis dan kualitas air. Hasil penelitian menunjukkan bahwa jumlah leukosit dan eritrosit berbeda secara signifikan antar perlakuan. Jumlah leukosit dan eritrosit pada nilem setelah 28 hari pemberian kalium diformat lebih tinggi daripada kontrol. Setelah uji tantang, jumlah leukosit meningkat hingga Hari ke-10 karena infeksi dan menurun ke kondisi normal pada Hari ke-14. Jumlah eritrosit menurun hingga Hari ke-10 mengindikasikan adanya aktivitas hemolitik A. hydrophila dan meningkat ke kondisi normal pada Hari ke-14. Gejala klinis setelah uji tantang relatif sama seperti hemoragik, ulser, exopthalmia, *abdominal dropsy,* dan berenang abnormal. Perlakuan penambahan kalium diformat 0,3% memiliki tingkat kelangsungan hidup tertinggi setelah uji tantang dibandingkan dengan perawatan lain (81,67%), memiliki jumlah leukosit dan eritrosit tertinggi selama infeksi, dan pulih lebih cepat daripada perawatan lain.

Kata kunci: *Aeromonas hydrophila*, Eritrosit, Imunostimulan, Leukosit, Tingkat kelangsungan hidup

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1. Introduction

Nilem carp (*Osteochilus hasselti*) is one of the freshwater fish commodities that are widely cultured by the community, especially in West Java (Mulyasari et al., 2010). The fish has high economic value and the eggs have the potential to become export commodities as a substitute for caviar (Subagja et al., 2006). Nilem carp is quite popular due to the tasty of meat and eggs (Pratiwi et al., 2011).

The market for nilem carp is quite extensive, ranging from traditional markets, restaurants to exports. Its economic value will increase if it has been processed and made into products such as jerky, fried baby fish, pindang, smoked and canned (Yandra et al., 2014). Along with the high market demand for nilem carp, it must be supported by increasing production from aquaculture. The main obstacle in aquaculture production is the occurrence of disease outbreaks (Mishra et al*.,* 2017). Disease outbreaks disrupt aquaculture productivity, even cause failures and economical losses for farmers (Kurniawan, 2012). Environmental factors which are not suitable for the aquaculture and decreased water quality may cause fish to be susceptible to disease (Rahmawati and Hartono, 2012). The bacteria that commonly infected freshwater fish are *Aeromonas hydrophila* and causes the Motile Aeromonas Septicemia (MAS) disease (Fernandez et al*.,* 2014). *A. hydrophila* infections in fish are characterized by small injuries on the body surface which resulted in loss of fish balance, darkening body colour, prominent eyes and bleeding (Noor El Deen, 2014). Therefore, there is a need for prevention efforts to anticipate the risk of fish disease infections. One solution to anticipate the disease is by increasing the fish resistance on pathogens through immunostimulant administration. Immunostimulant are biological and chemical compounds that can activate leukocytes to make fish more resistant to infection by viruses, bacteria, fungi and parasites (Raa, 2000). One of the immunostimulant that can be used is potassium diformate.

Potassium diformate (PD) as feed additive is categorised as dietary acidifier and proposed to replace the use of AGP (Antibiotic Growth Promoter). Organic acids and salts in potassium diformate can also contribute in ATP generation to produce energy in the body (Da Silva et al., 2013). The benefit of potassium diformate in commercial fish feed is that it can kill Gram-negative bacteria directly or have a bactericidal effect so that the mortality rate of the fish will be decreased (Kühlmann et al., 2011). Low pH value in potassium diformate may alter cell metabolism and enzyme activity, thus inhibiting intraluminal microbial growth, especially pathogenic ones. Some research has shown a reduction in the number of bacteria in stomach and intestines. However, Lactobacillus (lactic acid bacteria) which is beneficial was not affected, even growing in amount, so that it inhibits the pathogen (Lückstadt*,* 2008). Positive effect of potassium diformate on fish immune is not widely studied. This research aimed to determine the most effective dosage of potassium diformate additives in feed to increase the immune performance of nilem carp (*Osteochilus hasselti*)

2. Materials and Methods

2.1. Description of this Research

The materials used in this research include nilem fingerlings (average body length 5-7 cm) obtained from Ciparanje Freshwater Fisheries Area, Faculty of Fisheries and Marine Sciences, Universitas Padjadjaran; potassium diformate with brand name AQUAFORM (PT. Novindo Agritech Utama, South Tangerang, Indonesia); *A. hydrophila* isolates from Molecular Microbiology and Biotechnology Laboratory Faculty of Fisheries and Marine Sciences, Universitas Padjadjaran; and commercial feed (FF999 produced by PT Central Proteina Prima TBK, South Jakarta, Indonesia). This research was conducted in October to December 2018 at Hatchery in Ciparanje Freshwater Fisheries Area and Molecular Microbiology and Biotechnology Laboratory, Faculty of Fisheries and Marine Sciences, Universitas Padjadjaran. The method used was Completely Randomized Design (CRD) method with 4 treatments and 4 replications. Nilem fingerlings were kept in 16 aquariums with size of 40 x 30 x 30 cm^3 for 28 days. Each aquarium consisted of 20 nilem fingerlings and were fed with feed that had been given potassium diformate (PD) with different doses of 0 % PD (A); 0.1 % PD (B); 0.3 % PD (C) and 0.5 % PD (D). The feeding rate is 5% from biomass.

2.2. Mixing of PD in feed

Commercial feed and PD were weighed according to the treatment, then poured into a tray. Mixed them gently for 5 minutes until homogeneous and sprayed with water as much as 10% from total feed as a binder then dried in room temperature without exposed to direct sunlight.

2.3. Number of Leukocytes and Erythrocytes

Observation of leukocytes number and erythrocytes number was done six times; before treatment, 28th after PD treatment, the 3rd day after challenged test, the 7th day after challenged test, the 10th day after challenged test and the 14th day after challenged test. According to Blaxhall and Daisley (1973) the first step taken was to slice the base of the fish and then blood was sucked up to a scale of 0.5 with a thoma pipette. Then, added turk's solution for leukocyte to scale 11 and added hayem solution for erythrocyte to scale 101. Blood was homogenized then drip on the haemocytometer and closed with object glass.

2.4. Culture of *A. Hydrophila*

The isolates of *A. hydrophila* were inoculated on NA (Nutrient Agar) media using the scratch method to obtain a single colony, then incubated at 30 ºC for 24 hours. The results of the grew bacteria were taken by using one needle as much as 1 ose and dissolved with NB (Nutrient Broth) media. After that, the bacteria were incubated in a shaker incubator for 24 hours at 30 ºC at a speed of 150 rpm. Then the density calculation was performed to obtain a density of 10^8 cfu ml^{-1} using a spectrophotometer with a wavelength of 540 nm and an absorbent value reaching 0.8-1 for a density of 10^8 cfu ml^{-1}.

2.5. Challenged test with *A. hydrophila*

The infection of *A. hydrophila* against nilem carp was done intraperitoneally section with a density of 10^8 cfu ml^{-1}. Observation after challenged test was carried out for 14 days including leukocytes and erythrocytes number, survival rate and clinical symptoms. Observed clinical symptoms were body damage, fish feeding response, fish motion response and recorded daily.

2.6. Statistical Analysis

Data of leukocyte number, erythrocyte number and survival rate (before and post challenged test) were analyzed by F-test ANOVA with 95 % confidence level. The data which showed significantly different result will continue with Duncan's multiple distance test at the 95 % confidence level (Gaspersz, 1991). Data on macroscopic clinical symptoms and water quality were analyzed descriptively.

3. Results and Discussion

3.1. The Number of Leukocyte

The increase and decrease in the number of leukocytes can be used as an indication of the health condition in fish's body. Leukocytes play an important role in the fish defence system against pathogenic infections (Maryani et al., 2018). Leukocytes function as non-specific immune defenses that will localize and eliminate pathogens by phagocytosis (Anderson, 1974). The fish defence system will release neutrophils from blood vessels when an infection occurs, this can be caused by external chemical stimulation or chemotaxis (Vadstein, 1997). The average number of nilem fingerlings leukocytes is shown in Figure 1.

Based on Figure 1, the lowest increase in the average number of leukocytes occured in treatment A, while the highest increase occurred in treatment C. Normal leukocyte number of freshwater fish is ranged from 2.0 - 15.0 × 10^4 cells/mm^3 (Lagler et al*.,* 1977), then based on Hidayatullah's research results (2018) the number of nilem carp leukocytes is 6.48 - 7.17 × 10^4 cells/mm^3. The number of leukocytes after treatment increase until Day-28th showed higher numbers compared to nilem fingerlings fed with feed without potassium diformate additives ($p < 0.05$). The increasing number of leukocytes after feeding with potassium diformate was caused by organic acid which is made using dual salt technology to trigger an increase in the number of leukocytes.

The number of leukocytes after the challenged test varied with each treatment. The higher the potassium diformate dosage given does not always increase the number of leukocytes, this is presumably due to potassium diformate which is in the form of salt which may disrupt the osmoregulation system in fish. According to Lantu (2010) too many salt in fish body will cause more energy in fish bodies to stabilize the osmoregulation system.

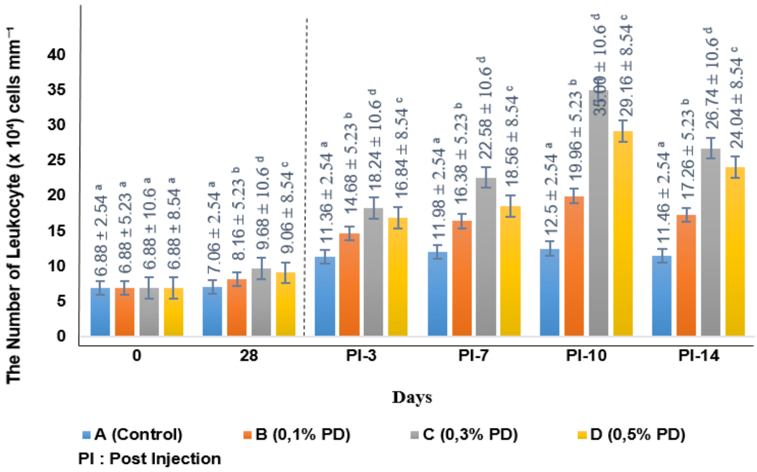

Figure 1. Leucocyte number of nilem (*Osteochilus hasselti*) during treatment period. Different superscript letters indicated significant differences. PD (Potassium diformate)

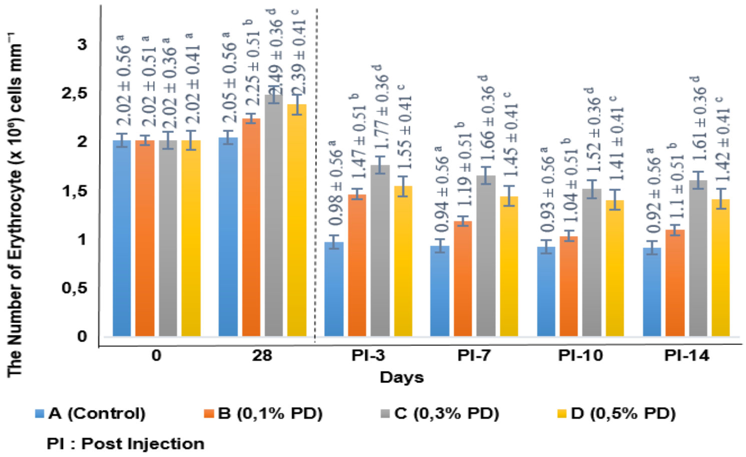

Figure 2. Erythrocyte number of nilem (*Osteochilus hasselti*) during treatment period. Different superscript letters indicated significant differences. PD (Potassium diformate)

The results showed that the average number of nilem fingerlings leukocytes in treatments A, B, C and D were increased in Day-3rd after challenged test due to the infection of *A. hydrophila*. This is supported by the research results of Shariff et al., (2001) which states that leukocyte counts increase after infection with *A. hydrophila* bacteria. The increase in the average number of leukocytes lasted until after Day-10th. However, the average number of nilem fingerlings leukocytes were decreased after Day-14th of challenged test. The highest decrease occurred in treatment C with a dose of 0.3 % potassium diformate which was equal to -23.60 %, while the lowest decrease occurred in treatment A by -8.32 %. The increasing number of leukocytes in nilem fingerlings showed that there was a disease caused by *A. hydrophila* infection while the decrease in the number of white blood cells is due to the ability of *A. hydrophila* to infect the nilem fish fingerlings on the 14th day begins to decline and the body of the fish experiences a healing period which is marked from the number of white blood cells decreasing towards normal. The number of leukocytes after challenged showed that potassium diformate additives in different doses of feed gave a significantly ($P < 0.05$).

3.2. The Number of Erythrocyte

Erythrocytes contain hemoglobin which functions in binding oxygen. The number of erythrocytes is influenced by fish activity, water temperature, type, age, gender and nutritional status (Witeska, 2013). The average number of erythrocytes in nilem fingerlings is shown in Figure 2.

Based on Figure 2, the lowest increase in the average number of erythrocytes occurred in treatment A which is equal to 2.05×10^6 cells/mm^3, while the highest increase occurred in treatment C, which is equal to 2.49×10^6

cells/mm^3 ($P < 0.05$). The number of erythrocytes in teleostei fish generally ranges from 1.00 - 3.00 × 10^6 cells / mm^3 (Irianto, 2005). The increasing amount of erythrocytes will increase the hemoglobin. Therefore, more oxygen can be bound. The results showed that nilem fingerlings in all treatments experienced a decrease in the number of erythrocytes which was quite drastic, however, the number of erythrocytes in treatment B, C and D were relatively higher compared to treatment A. This is presumably due to the increasing amount of leukocytes in nilem fingerlings body as a result of the increasing in body resistance due to potassium diformate addition. Therefore, lower level infection in treatments B, C and D resulted the internal organs such as the liver, spleen and spinal cord still functioning to form erythrocyte. Something similar found in the Sukenda et al., (2018) that the number of erythrocytes in tilapia fingerlings has decreased when infected by *A. hydrophila* bacteria. According to Amlacher (1970), apart from bacterial infection, the response to food can also influence the blood composition, including the number of erythrocytes which also affect the hematocrit. The number of erythrocytes of nilem fingerling from treatment A were decreased by -1.08 % on Day-14th of challenged test while the other treatment experienced an increase. The biggest increase was found in treatment C with 0.3 % dose of potassium diformate (5.59 % increase in erythrocytes number). The increasing number of erythrocytes towards normal on Day-14th after the challenged test showed that nilem fingerlings was in the healing period.

3.3. Survival Rate

Survival rate is a comparison between the number of living organisms at the end of a period and the number of living organisms at the beginning of the period (Effendie, 1997). The average survival rate of the treatment period is shown in Figure 3.

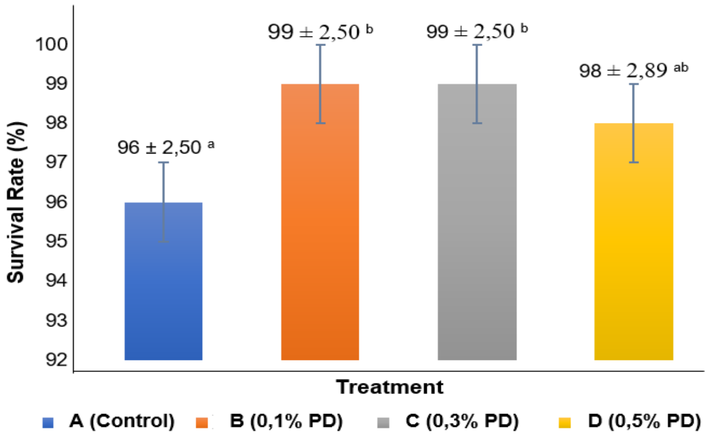

Figure 3. Survival rate of the nilem (*Osteochilus hasselti*) during treatment period. Different superscript letters indicated significant differences. PD (Potassium diformate)

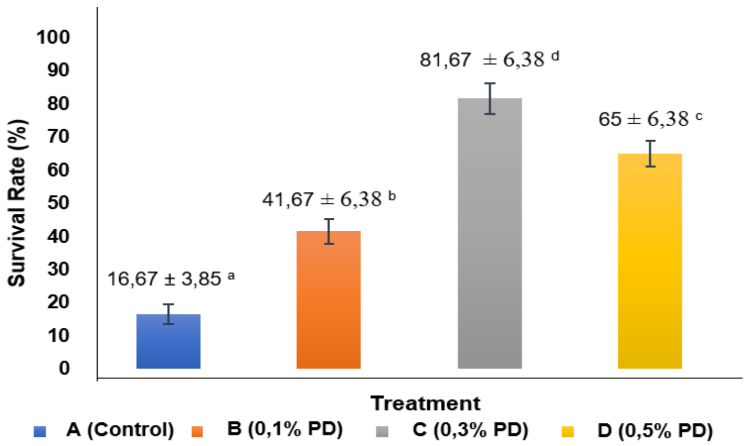

Figure 4. Survival rate after 14th days of challenged test

Based on Figure 3, nilem fingerlings from all treatments have high survival rate, however, nilem fingerlings which given potassium diformate as feed additives have higher survival rate. The average survival rate of treatment A was 96 %, treatment B and treatment C were 99 % and treatment D was 98 %. The F test results ($p < 0.05$) toward survival rate showed that the addition of potassium diformate in treatments B and C were significantly different from treatment A but not significantly different from treatment D. The results of the average survival rate after 14th days of challenged test is presented in Figure 4.

Based on Figure 4, it can be seen that survival rate of nilem fingerlings after challenged test were varied in each treatment ($P < 0.05$). The highest survival rate occurred in treatment C (81.67 %). Therefore, higher dosage of potassium diformate addition in feed does not always increase the survival rate of nilem fingerlings. Too many potassium diformate, which is in the form of salts, in the body of the fish will disturb the osmoregulation system.

Previous studies on tilapias showed that red tilapia which fed with potassium diformate and challenged by *Streptococcus agalactiae* showed lower mortality rate compared to control fish (Ng et al., 2009). It is in agreement with Elala and Ragaa (2015) which found a decrease in tilapia mortality rate (*A. hydrophila* challenge) since there is an increase in phagocytic activity and serum lysozyme of the treated group with concentration of 0.3% potassium diformate.

3.4. Macroscopic Clinical Symptoms

3.4.1. Body Damage

Based on the results of observations, nilem fingerlings body had shown the damage at the 6th hour after injection. Damage was seen at the body surface of nilem fingerlings which had been infected by *A. hydrophila*. Other symptoms which also occurring when infected by these bacteria were round or irregular ulcers with grayish red color and inflammation in the abdominal cavity (Noga, 2013). Damage on nilem fingerlings at the first 24th hours for each treatment during the challenged test were shown in Table 1.

Based on Table 1, body damage was first seen in treatment A in the form of hemorrhagic and ulceration. Clinical symptoms which appeared on nilem fingerlings in each treatment were not evenly distributed. This was presumably due to different body resistance of each individual. According to Irianto (2005), body resistance is determined by the type of fish, age, sex and nutrition. Body damage of nilem fingerlings in each treatment after challenged test are shown in Table 2.

Based on Table 2, body damage has begun to be found on Day-1st after challenged test. According to Most et al., (2002) in Wahjuningrum et al., (2013), *A. hydrophila* bacteria has optimal growth in the exponential phase which occurred at the first 4th - 12th hour. This showed that the *A. hydrophila* infection have multiplied and infected nilem fingerlings on 3rd hour after challenged test Day-1st. However, clinical symptoms began to appear after 6th hour. According to Haditomo et al., (2014) *A. hydrophila* bacteria has optimal growth in the exponential phase which occurs at the 16th - 22nd hours.

Table 1. Body damage at the first 24th hours for each treatment after challenged test

Hours	Treatment			
	A (Control)	**B (0.1% PD)**	**C (0.3% PD)**	**D (0.5% PD)**
3	-	-	-	-
6	abd	Ad	ad	A
9	abd	Abd	ad	Abd
12	abd	Abd	abd	Abd
15	abd	Abd	abd	Abd
18	abd	Abd	abd	Abd
21	abd	Abd	abd	Abd
24	abd	Abd	abd	Abd

Description: a (hemorrhagic), b (ulceration), c (exopthalmia) and d (dropsy)

Table 2. Damage of nilem fingerlings in each treatment after challenged test

Days-	Treatment			
	A (Control)	B (0.1% PD)	C (0.3% PD)	D (0.5% PD)
1	abd	Ad	ad	Ad
2	abcd	Abd	ad	Abd
3	abcd	Abd	ad	Abd
4	abcd	Abd	abd	Abcd
5	abcd	Abd	abd	Abcd
6	abcd	Abd	abd	Abcd
7	abcd	Abcd	abd	Abcd
8	abcd	Abcd	abd	Abcd
9	abcd	Abd	abd	Abcd
10	abcd	Abd	ab	Abcd
11	abcd	Abd	ab	Abd
12	abcd	Ab	ab	Abd
13	cbd	Ab	a	Ad
14	cbd	Ab	a	Ad

Description: a (hemorrhagic), b (ulceration), c (exopthalmia) and d (dropsy)

Observation of challenged test Day-2nd was performed in every 8 hours. Treatment A, showed clinical symptom in the form of exopthalmia (Figure 5). According to Olga (2012) fish infected with *A. hydrophila* bacteria experience macroscopic clinical symptoms such as loss of appetite, injuries on the body surface, bleeding in the gills, dropsy, ulceration and if surgery was performed there will be swelling and damage to the liver, kidney and spleen. Mangunwardoyo et al., (2010) also states that fish infected with *A. hydrophila* shows pathological changes such as body color turned dark, weak, unresponsive to both feed and shock and local bleeding in the body.

A. hydrophila will excrete exotoxin and endotoxin compounds. Endotoxin compounds are consisted of hemolysin, protease, enteroxin, lechitinase and leucocidine. Substances of hemolysin and lechitinase can lyse erythrocytes and destroy various tissue cells. Exotoxins released by *A. hydrophila* will be spread throughout the body through the bloodstream, causing hemolysis and rupture of blood vessels. Rupture in blood vessels and tissues will result in bleeding out of the veins causing hemorrhagic on the body surface.

Damage on the body's surface is getting worse on Day-3rd to Day-9th. Nilem fingerlings from treatment A showed hemorrhagic, exopthalmia, ulceration (Figure 5) and dropsy. Hemorrhagic are the first reaction from the *A. hydrophila* infection which causes damage to the tissue. Dropsy is caused by the release of Aerolysin Cytotoxic Enterotoxin (ACT-gene) which can cause tissue damage (Indriani et al*.,* 2014). According to Noor El Deen et al*.,* (2014), *A. hydrophila* infection in fish is characterized by small injuries on the body surface which results in loss of fish balance, darkening body color, exopthalmia and bleeding. The damage on the body surface experienced by nilem fingerling after challenged test can be seen in Figure 5.

After Day-14th the body damages are tend to heal in potassium diformate treatments, this is due to potassium diformate can improve the general health status of cultured animals by its stronger antimicrobial effect towards coliform bacteria, *Escherichia coli* and *Salmonella* sp., than towards lactobacilli (Elala & Ragaa, 2015). It also reported that the total bacteria per gram of faeces was significantly reduced in the fish fed with an organic acid blend and potassium diformate as feed additives (Ng et al., 2009). Similarly, Da Silva et al., (2013) found that organic acids (butyrate, propionate and acetate) have the highest inhibitory activity against vibrio species in marine shrimp. These acids can penetrate through the membrane of Gram-negative bacteria and release protons into the cytoplasm. Thus, the bacteria consume a large amount of ATP to excrete protons in trying to maintain a balanced intracellular pH, resulting in the depletion of cellular energy with eventual cell death (Defoirdt et al., 2009).

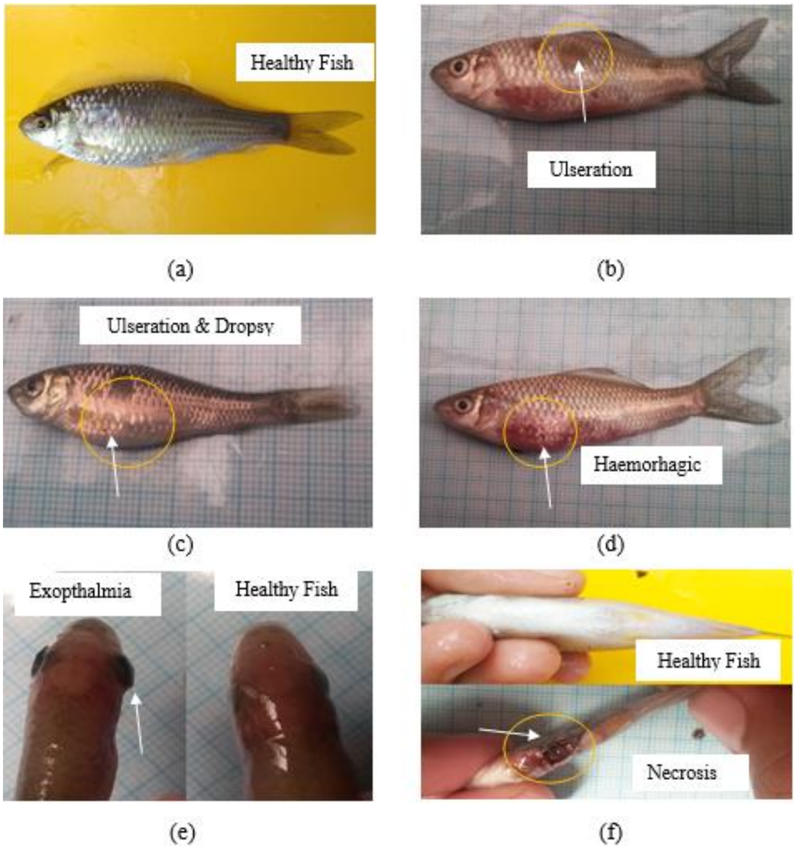

Figure 5. Body damage: (a) Healthy fish, (b) Ulceration, (c) Dropsy & Ulceration, (d) Hemorrhagic, (e) Comparison of exopthalmia & healthy fish and (f) Comparison of necrosis & healthy fish

3.4.2. Fish Feeding Response

Feeding response of nilem fingerlings infected by *A. hydrophila* were observed every day. Fish infected with *A. hydrophila* showed signs of abnormal fish behavior, swim slowly, gasp at the surface of the water and low response to feed (Kabata, 1985). Feeding response of nilem fingerlings can be seen in Table 3.

Based on Table 3, it can be seen that, in challenged test Day-1st, nilem fingerlings from all treatment showed a normal feeding response. Nilem fingerlings from treatment A has shown a moderate feeding response on Day-2nd and low feeding response on Day-3rd to Day-11th. Decrease in feeding response also happened in other treatments. Nilem fingerlings of treatment B and D showed a decreasing feeding response in Day-3rd to Day-7th. Meanwhile, feeding response of nilem fingerlings from treatment C started to decrease from Day-3rd to Day-5th, however, feeding response started to increase on Day-6th. According to Muslikha et al*.,* (2016), the symptoms of fish infected with *A. hydrophila* bacteria varies, from sudden death, lack of appetite, abnormal swimming movement, pale gills, flatulence and ulcerated skin. Ulcerated skin can occurs in any part of the fish and often surrounded by red tissue. Other organs that are often affected by this disease are bile, digestive tract, liver, kidney.

In Day-8th the feeding response in all potassium diformate treatments was returned to normal condition, this is due to the release of H^+ ions by potassium diformate which made acid condition (low pH level) in the digestive tract (Yustiati et al., 2019; Nugraha et al., 2020). Similar results also found by Kirchgessner et al., (1992), who reported that formic acid (in this case potassium diformate) can reduce Gram-negative bacteria and increased lactic acid bacteria (LAB). LAB can proliferate at a relatively low pH condition, which means that they are more resistant to potassium diformate than Gram-negative bacteria. Furthermore, colonization of LAB inhibited pathogenic bacteria attachment and proliferation in the digestive tract.

Table 3. Feeding response of nilem fingerlings

Days-	Treatment			
	A (Control)	**B (0.1% PD)**	**C (0.3% PD)**	**D (0.5% PD)**
1	+++	+++	+++	+++
2	++	+++	+++	+++
3	+	++	++	++
4	+	++	++	++
5	+	++	++	++
6	+	++	+++	++
7	+	++	+++	++
8	+	+++	+++	+++
9	+	+++	+++	+++
10	+	+++	+++	+++
11	+	+++	+++	+++
12	++	+++	+++	+++
13	++	+++	+++	+++
14	++	+++	+++	+++

Description: (+) Low response to feed, (++) Moderate response to feed and (+++) High response to feed.

In Day-8th the feeding response in all potassium diformate treatments was returned to normal condition, this is due to the release of H^+ ions by potassium diformate which made acid condition (low pH level) in the digestive tract (Yustiati et al., 2019; Nugraha et al., 2020). Similar results also found by Kirchgessner et al., (1992), who reported that formic acid (in this case potassium diformate) can reduce Gram-negative bacteria and increased lactic acid bacteria (LAB). LAB can proliferate at a relatively low pH condition, which means that they are more resistant to potassium diformate than Gram-negative bacteria. Furthermore, colonization of LAB inhibited pathogenic bacteria attachment and proliferation in the digestive tract.

3.4.3. Fish Motion Response

Fish swimming movement response toward shock was observed for 14th days after injection of *A. hydrophila*. According to Olga (2012), fish which is infected with the *A. hydrophila* will have a decreasing swimming movement and tend to swim on the surface of the water. This is due to damage to the fins which affects swimming movements to become unstable. The results of observations toward swimming response of nilem fingerlings infected by *A. hydrophila* are shown in Tabel 4.

Table 4. Swimming response of nilem fingerlings infected by *A. hydrophila*

Days-	Treatment			
	A (Control)	**B (0.1% PD)**	**C (0.3% PD)**	**D (0.5% PD)**
1	++	++	++	++
2	++	++	++	++
3	+	++	++	++
4	+	++	++	++
5	+	++	++	++
6	+	++	++	++
7	+	++	++	++
8	+	++	++	++
9	+	++	++	++
10	+	++	++	++
11	+	++	++	++
12	+	++	++	++
13	+	++	++	++
14	+	++	++	++

Description: (+) Motion response is not normal and (++) Motion response is normal

Table 5. Water quality observations

Treatment	Parameter		
	Temperature (^oC)	**pH**	**Dissolved Oxygen (DO) (mg L^{-1})**
A (Control)	24.3 - 25	7.58 – 7.75	5.9 – 6.7
B (0.1% PD)	24.2 - 25	7.59 – 7.75	5.9 – 6.7
C (0.3% PD)	24.2 - 25	7.55 – 7.71	5.9 – 6.5
D (0.5% PD)	24.3 – 25.1	7.55 – 7.71	5.8 – 6.5
Standard	20-28 (Serdiati, 1988)	6.5 – 8.5 (SNI)	≥ 5 (SNI)

Based on Table 4, nilem fingerlings from all treatment had a normal movement response on challenged test Day-1st after *A. hydrophila* injection. However, nilem fingerlings from treatment A began to show changes in swimming movement such as slow response, moving towards the oxygen source, staying at the bottom of the aquarium and having no motion response toward the shock stimulant. This is allegedly due to the damage on the body surface and lack of nutrient intake.

Nilem fingerlings in treatment B, C and D showed normal motion response after challenged test. The fish showed agile movement and respond to the shock stimulant.

3.5. Water Quality

Bad water quality may cause the stress on fish. Therefore, the water quality in this research was well maintained to prevent stress on fish before the challenged test. Observation of water quality was carried out six times (weekly) during the research (from the first day of treatment until the end of the challenged test), includes measurement of temperature, acidity (pH) and dissolved oxygen (DO) levels. The results of water quality observations during the research can be seen in Table 5.

The results of the observations in Table 5 showed that water quality measurements during research still meet the existing standards. The average temperature measurement values for each treatment ranged from 24.2 - 25.1 ºC, the average pH measurements ranged from 7.55 - 7.75 and the average DO measurements ranged from 5.8 - 6.7 mg L^{-1}. These results showed that the addition of potassium diformate in treatments B, C and D did not affect water quality which is still met the standards in accordance with Serdiati (1988) and the Indonesian National Standard (SNI) in 1999 for cultured carp.

4. Conclusion

Based on the results, it can be concluded that potassium diformate addition with a dose of 0.3% is the most effective to increase the immune performance of nilem fingerlings which can be seen from the increase in the number of leukocytes and erythrocytes after 28 days of treatments. After being challenged by *A. hydrophila* for 14 days there was a decrease in the number of leukocytes by -23.60%, an increase in the number of erythrocytes by 5.92% and a survival rate of 81.67% while survival rate in control was only 16.67%.

References

Amlacher, E. 1970. Textbook of Fish Disease. New York, TFH Publications, Inc., 302pp.

Anderson, D.P. 1974. Fish Immunology: Book 4. Neptune City, New Jersey, United States, TFH Publications, Inc., 239pp.

Aoki, T. 1999. Motile Aeromonas (*Aeromonas hydrophila*). In: Woo, P.T.K., Bruno, D.W. (Eds.). Fish Diseases and Disorders Volume 3: Viral, Bacterial and Fungal Infections, London, England, CABI Publishing., 930pp.

Blaxhall, P.C., & Daisley, K.W. (1973). Routine Haematological Methods for Use with Fish Blood. *Journal of Fish Biology*, 5(6), 771-781. http://doi.org/10.1111/j.1095-8649.1973. tb04510.x

Da Silva, B.C., Vieira, F.D.N., Mouriño, J.L.P., Ferreira, G.S., & Seiffert, W.Q. (2013). Salts of organic acids selection by multiple characteristics for marine shrimp nutrition*. Aquaculture,* 384(387), 104-110.
doi :10.1016/j.aquaculture.2012.12.017.

Defoirdt,T., Boon, N., Sorgeloos, P., Verstraete, W., & Bossier, P. 2009. Short-chain fatty acids and poly-beta-hydroxyalkanoates: (New) Biocontrol agents for a sustainable animal production. *Biotechnol Adv.,* 27(6), 680-685.

doi: 10.1016/j.biotechadv.2009.04.026.

Effendie, M.I. 1997. Aquatic Biology. Yogyakarta, Indonesia, Yayasan Pustaka Nusatama., 163 pp.

Elala, N.M.A., & Ragaa, N.M. 2015. Eubiotic effect of a dietary acidifier (potassium diformate) on the health status of cultured *Oreochromis niloticus*. *Journal of Advance Research*, 6, 621-629. http://dx.doi.org/10.1016/j.jare.2014.02.008

Fernandez, J.B., Yambot, A.V., & Almeria, O. 2014. Vaccination of Nile tilapia (*Oreochromis niloticus*) using lipopolysaccharide (LPS) prepared from *Aeromonas hydrophila*. *International Journal of Fauna and Biological Studies,* 1(4), 1-3.

Gaspersz, V. 1991. Experimental Design Method. Bandung, Indonesia, CV. Armico., 472 pp.

Haditomo, A.H.C., Widanarni., & Lusiastuti, A.M. 2014. Development of *Aeromonas hydrophila* in Various Culture Media, *Proceedings of the third annual National Seminar on Fisheries and Marine Research Results* (pp. 357-364). Semarang, Indonesia, Undip. 549pp.

Hidayatullah, A., Rosidah, Subhan, U., & Suryadi, I.B.B. 2018. Hematology Parameters of Nilem Padjadjaran Strain (*Osteochilus* Sp.) Infected by *Aeromonas hydrophila. Global Science Journal,* 6(7), 322-329.

Indriani, A.D., Prayitno, S.B., & Sarjito. 2014. The Utilization of Red Ginger (*Zingiber officinale* var. Rubrum) Extract as Alternative Treatment for Tilapia (*Oreochromis niloticus*) Infected by *Aeromonas hydrophila*. *Journal of Aquaculture Management and Technology*, 3(3), 58-65.

Irianto, A. 2005. Fish Pathology Teleostei. Yogyakarta, Indonesia, UGM Press., 265 pp.

Kühlmann, K.J., Jintasataporn, O., & Lückstadt, C. 2011. Effect of dietary potassium diformate (KDF) on survival of juvenile white-leg shrimp, *Litopenaeus vannamei*, challenged with *Vibrio harveyi* under controlled conditions. *Internasional Aquafeed*, March–April, 19–22.

Kabata, Z. 1985. Parasites and Disease of Fish Cultureed in the Tropics. (1st edition). Taylor & Francis, London and Philadelphia. 318pp.

Kirchgessner, M., Gedek, B., Wiehelr, S., Bott, A., Eidelsburger, U., & Roth, F.X. (1992). Influence of formic acid, calciumformate and sodiumhydrogencarbonate on the microflora in different segments of the gastrointestinal tract. Communication. Investigations about the nutritive efficacy of organic acids in the rearing of piglets. *J. Anim. Physiol. Anim. Nutr.,* 68, 73-81.

Kurniawan, A. 2012. Aquatic Disease. Pangkalpinang, Indonesia, UBB Press, 225pp.

Lagler, K.F., Bardach, J.E., Miller, R.R., & Passino, D.R.M. 1977. Ichthyology. United State, John Willey & Sons Inc., 528pp.

Lantu, S. 2010. Osmoregulation in Aquatic Animals. *Jurnal Perikanan dan Kelautan,* 6(1), 46-50.

Lückstadt, C. 2008. The Use Of Acidifiers In fish Nutrition. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources* 3(44),1-8. http://doi.org/10.1079/PAVSNNR20083044

Maryani., Monalisa, S.S., Rosita, Rozik, M., & Pratasik, S.B. 2018. Effectivity of *Arcangelisia flava* as immunostimulant to prevent *streptococcosis* on Nile tilapia, *Oreochromis niloticus. AACL Bioflux,* 11(6), 1834-1843.

Mangunwardoyo, W., Ratih, I., & Etty, R. 2010. Pathogenicity and virulency of *Aeromonas hydrophila* stainer on nila fish (*Oreochromis niloticus* Lin.) using koch postulate. *Jurnal Riset Akuakultur*, 5(2), 245-255. http://doi.org/10.15578/jra.5.2.2010.145-255

Mishra, S.S., Rakesh, D., Dhiman, M., Choudhary, P., Debbarma, J., Sahoo, S.N., Barua, A., Giri, B.S., Ramesh, R., Ananda, K., Mishra, C.K., & Swain, P. 2017. Present Status of Fish Disease Management in Freshwater Aquaculture in India: State-ofthe-Art-Review. *J. Aquac Fisheries,* 1(003), 1-9. http://doi.org/10.24966/AAF-5523/100003

Mulyasari, M., Soelistyowati, D.T., Kristanto, A.H., & Kusmini, I.I. 2010. Characteristic genetic of six population of nilem carp fish at West Java. *Jurnal Riset Akuakultur*, 5 (2), 172-182. http://doi.org/10.15578/jra.5.2.2010.175-182

Muslikha., Pujiyanto, S., Jannah, S.N., & Novita, H. 2016. Isolation, Characterization of *Aeromonas hydrophila* and Detection of Genes Causes of Disease *Motile Aeromonas Septicemia* (MAS) with 16s of RNA and Aerolysin in Catfish (*Clarias* Sp.). *Jurnal Biologi,* 5(4), 1-7.

Ng, W.K., Koh, C.B., Sudesh, K., & Zahrah, A.S. 2009. Effects of dietary organic acids on growth, nutrient digestibility and gut microflora of red hybrid tilapia, *Oreochromis* sp., and subsequent survival during a challenge test with *Streptococcus agalactiae. Aquaculture Research,* 40(13), 1490-1500. https://doi.org/10.1111/j.1365-2109.2009.02249.x

Noga, E.J. 2013. *Fish Disease: Diagnosis and Treatment 2nd edition.* United State, Wiley Blackwell., 536pp.

Noor El Deen, A.E., Sohad, Dorgham, M., Hassan, A.H.M., & Hakim, A.S. 2014. Studies on *Aeromonas hydrophila* in Cultured *Oreochromis niloticu*s at Kafr El Sheikh Governorate, Egypt with Reference to Histopathological Alterations in Some Vital Organs. *World Journal of Fish and Marine Sciences,* 6 (3), 233-240. https://doi.org/10.5829/idosi.wjfms.2014.06.03.83136

Nugraha, A.A., Yustiati, A., Bangkit, I., & Andriani, Y. 2020. Growth performance and survival rate of giant gourami fingerlings (*Osphronemus goramy* Lacepede, 1801) with potassium diformate addition. *World Scientific News,* 143,103-114.

Olga. 2012. The Pathogenicity of Aeromonas hydrophila ASB01 on Snakehead (*Ophicephalus striatus*). *Sains Akuatik,* 14(1), 33–39.

Pratiwi., Rostika, R., & Dhahiyat, Y. 2011. The Effect of Feed Level on the Growth and Deposition Rate of Heavy Metals in Nilem Fish in the Cages of Floating Net of Ir. H. Djuanda. *Jurnal Akuatika,* 2(2), 1-11.

Raa, J. 2000. The use of immunostimulants in fish and shellfish feeds. In: Cruz -Suárez, L.E., Ricque-Marie, D., Tapia-Salazar, M., Olvera-Novoa, M.A. y Civera Cerecedo, R., (Eds.). Avances en Nutrición Acuícola V. Mexico, Memorias del V Simposium Internacional de Nutrición Acuícola.

Rahmawati, H., & Hartono, D. 2012. Strategy for Developing Cultured Freshwater Fish Business. *Naturalis,* 1(2), 129-134.

Serdiati. 1988. Pengaruh Padat Penebaran Terhadap Pertumbuhan Ikan Mas (*Cyprinus carpio*) yang Dipelihara dalam Karamba pada Kolam dengan Input Air Limbah Rumah Tangga. Skripsi. Universitas Hassanudin.

Shariff, M., Jayawardena, P.A., Yusoff, F.M., & Subasinghe, R. 2001. Immunological parameters of Javanese carp *Puntius gonionotus* (Bleeker) exposed to copper and challenged with *Aeromonas hydrophila*. *Fish Shellfish Immunol*, 11(4), 281-91. https://doi.org/10.1006/fsim.2000.0309

SNI 01-6133–1999. 1999. Carp Production (*Cyprinus carpio* Linneaus) strain Majalaya Fingerlings Spread Class. Indonesia, BSN., 11pp.

Subagja, J., Gustiano, R., & Winarlin, L. 2006. Preservation of Nilem Carp (*Osteochilus hasselti* C.V) Through Hatchery Technoogy. *Proceedings of the National Workshop on Management and Protection of Genetic Resources in Indonesia,* 279-286.

Sukenda L., Jamal., Wahjuningrum, D., & Hasan, A. 2008. Use of Chitosan to Prevent *Aeromonas hydrophila* Infection on Catfish *Clarias* sp. *Jurnal Akuakultur Indonesia*., 7(2), 159-169. https://doi.org/10.19027/jai.7.159-169

Sukenda, S., Romadhona, E.I., Yuhana, M., Pasaribu, W., & Hidayatullah, D. 2018. Efficacy of whole-cell and lipopolysaccharide vaccine of *Aeromonas hydrophila* on juvenile tilapia *Oreochromis niloticus* against *Motile Aeromonas Septicemia*. *AACL Bioflux,* 11(5), 1456-1466.

Vadstein O., 1997. The use of immunostimulation in marine larviculture: possibilities and challenges. *Aquaculture,* 155(1-4),401-417. https://doi.org/10.1016/S0044-8486(97)00114-2

Wahjuningrum, D., Retno, A., & Mia, S. 2013. Prevention of *Aeromonas hydrophila* on catfish juvenile using garlic and shatterstone herb. *Jurnal Akuakultur Indonesia,*12(1),86-94. https://doi.org/10.19027/jai.12.86-94

Witeska, M. 2013. Erythrocytes in teleost fishes: a review. *Zoology and Ecology,* 23(4),275-281. https://doi.org/10.1080/21658005.2013.846963

Yandra, E., Nuraini, & Alawi, H. 2014. Hybridization of Gold Fish *(Carassius Auratus Auratus)* With Nilem *(Osteochillus Hasselti). JOMFAPERIKA,* 1(2), 1-8.

Yustiati, A., Kundari, D.F., Suryana, A.A.H., & Suryadi, I.B.B. 2019. Effectiveness of potassium diformate addition to feed to improve immune system of Pangasius (*Pangasianodon hypophthalmus*) that challenged by *Aeromonas hydrophila*. *World Scientific News,* 134(2), 86-100.