The potential of pineapple peel flour (*Ananas comosus*) in controlling *Aeromonas hydrophila* infection in sangkuriang catfish (*Clarias gariepinus*)

Ita Sari* | Agustina Agustina | Ricko Reynalta

Department of Aquaculture, Faculty of Fisheries and Marine Science, Mulawarman University
Jl. Gunung Tabur No. 1. Kampus Gn. Kelua Samarinda 76123
**E-mail: itasari512@gmail.com*

ARTICLE INFO

Research Article

Article history:
Received April 24, 2024
Received in revised form September 20, 2024
Accepted September 15, 2024

DOI: 10.30872/jipt.v4i1.1252

Keywords: *Aeromonas hydrophila, catfish, pineapple peel, hematological parameters, survival rate*

ABSTRACT

Hematological conditions can reflect the health status of fish. This study aimed to analyze the effect of adding different doses of pineapple peel flour to feed on clinical symptoms, anatomical pathology, survival rate, hematological parameters, and cellular immunity of Sangkuriang catfish after infection with Aeromonas hydrophila. The study was conducted using a Completely Randomized Design (CRD) with four treatments and three replications. The treatments consisted of feed supplemented with 0%, 10%, 20%, and 30% pineapple peel flour. The results showed that different doses of pineapple peel flour in feed had a significant effect (P<0.05) on anatomical pathology, survival rate, hematocrit levels, total erythrocytes, total leukocytes, and the percentage of lymphocytes, monocytes, and neutrophils in Sangkuriang catfish after A. hydrophila infection. The findings also indicated that the 20% dose of pineapple peel flour in feed was the most effective in controlling A. hydrophila infection.

INTRODUCTION

Sangkuriang catfish (*Clarias gariepinus*) is a highly popular freshwater fish widely favored by the public. This species has several advantages, including rapid growth and adaptability to various environmental conditions, making it suitable for cultivation even in limited land and water resources. Additionally, catfish exhibit high disease resistance. However, this does not mean they are completely immune to infections or diseases (Raihan, 2023).

One of the major diseases affecting catfish is Motile Aeromonas Septicemia (MAS), caused by the bacterium Aeromonas hydrophila (Widyawati et al., 2020). A. hydrophila is an opportunistic pathogen, meaning it attacks when the host's immune system is weakened. This Gram-negative bacterium can cause high mortality rates in fish, reaching 80–100% within a short period (Muslikha et al., 2016). The infection often occurs when fish are stressed or already weakened by parasitic infections.

Several feed additives have been studied for their potential to prevent fish diseases and enhance production. One promising source of feed additives is fruit peel waste, which remains underutilized despite its nutritional benefits. Pineapple peel, in particular, contains dry matter (88.95%), crude protein (8.78%), crude fiber (17.09%), crude fat (1.15%), ash (3.82%), and nitrogen-free extract (BETN) (66.89%) (Nurhayati, 2013). Moreover, pineapple peel and core extract contain phytochemical compounds such as saponins, flavonoids, tannins, vitamin C, and bromelain enzyme, which provide various health benefits (Sumiati, 2020).

The use of pineapple peel waste as a feed additive has been tested in fish. For instance, incorporating 30% pineapple peel into feed has been shown to enhance growth rates in Nile tilapia (Sukri et al., 2022). Additionally, Van Doan et al. (2021) found that 10 g/kg of pineapple peel flour in feed was the optimal dosage for controlling Streptococcus agalactiae infection in Nile tilapia. Utilizing this waste not only benefits aquaculture but also helps reduce organic waste in the environment.

Based on these findings, this study aims to evaluate the potential of pineapple peel flour in controlling A. hydrophila infection in Sangkuriang catfish. The study focuses on clinical symptoms, anatomical pathology, survival rate, and hematological parameters in C. gariepinus after bacterial infection.

METHODOLOGY

This research was conducted from September to November 2023, covering preparation, maintenance, and observation of hematological parameters. The study was carried out at the Microbiology and Aquaculture Biotechnology Laboratory and the Fish Nutrition Laboratory, Faculty of Fisheries and Marine Science, Mulawarman University.

Research design

The research utilized a laboratory experimental method with a Completely Randomized Design (CRD). The dosage treatment of pineapple peel flour was based on the study conducted by Sukri et al. (2022). This study used four treatments, each consisting of three replications. The treatments applied in this study were as follows:

P0 = Control, without pineapple peel flour
P1 = Feed with the addition of 10% pineapple peel flour
P2 = Feed with the addition of 20% pineapple peel flour
P3 = Feed with the addition of 30% pineapple peel flour

Data collection

Clinical symptoms before the challenge test were observed on days 10 and 30, while clinical symptoms and anatomical pathology observations after fish were challenged with *Aeromonas hydrophila* were conducted from days 35 to 45. Fish survival rate was calculated using the formula from Effendie (2002) as follows:

$$\textbf{Survival Rate (\%)} = \frac{Nt}{N0} \times 100\%$$

Where:

SR = Fish survival rate (%)
Nt = Number of fish at the end of the study
N0 = Number of fish at the beginning of the study

Hematological parameters were collected on days 0, 20, 37, and 45 and calculated using the differential leukocyte count formula from Rahma et al. (2015). Supporting parameters included water quality parameters such as temperature, pH, dissolved oxygen, and ammonia. Temperature was measured daily, pH and dissolved oxygen were measured every seven days, and ammonia was measured at the beginning, middle, and end of the study.

Data analysis

The collected data on clinical symptoms, anatomical pathology, and water quality were analyzed descriptively. Meanwhile, fish survival rate, hematological parameters, and cellular immune response of catfish were statistically tested using Analysis of Variance (ANOVA) at a 95% confidence level. If a significant effect was found, Duncan's Multiple Range Test (DMRT) was conducted as a post hoc test.

RESULT AND DISCUSSION

Clinical symptoms and anatomical pathology

The clinical symptoms of *Sangkuriang* catfish observed on days 10 and 30, before the challenge test with *Aeromonas hydrophila*, showed normal activity, swimming patterns, reflex responses, and appetite across all treatments. Changes in clinical symptoms in the P0 (control) group were observed after the fish were challenged with *A. hydrophila* on day 36. The fish remained mostly at the bottom of the container, with reduced reflex response to touch. Between days 37 and 39, some fish stayed at the bottom, while others swam near the surface, exhibited reduced reflexes, and showed decreased appetite. Five fish died during this period. By days 40–45, surviving fish in the control group displayed normal clinical symptoms. In the P1 (10% pineapple peel flour) group, from days 36 to 39, fish remained at the bottom and showed reduced appetite, with one fish dying. On days 40–43, fish exhibited decreased reflex responses when the aquarium was touched, along with continued reduced appetite. However, by days 44–45, the fish began to recover and appeared normal. For the P2 (20% pineapple peel flour) group, between days 36 and 39, some fish stayed at the bottom, while others swam near the surface with a slight decrease in appetite. From days 40–43, some fish remained at the bottom, but their appetite began to normalize. By days 44–45, most fish had recovered and displayed normal clinical symptoms. In the P3 (30% pineapple peel flour) group, clinical symptoms between days 36 and 39 included fish staying at the bottom of the container, some swimming near the surface, and reduced appetite. Between days 40 and 43, some fish remained at the bottom, but their appetite showed improvement. By days 44–45, all fish exhibited normal clinical symptoms.

Fish experiencing stress or health disturbances due to infectious or non-infectious diseases often undergo physiological changes, including behavioral alterations, swimming patterns, reflex responses, and appetite reduction (Saptiani, 2009). This aligns with observations in the control group, where fish exhibited erratic swimming behavior and loss of balance before dying. Triyaningsih et al. (2014) stated that clinical symptoms of *A. hydrophila* infection in fish include reduced feeding response, abnormal swimming movements, and bodily wounds. Similarly, a study by Mulia (2010) reported both internal and external symptoms in fish infected with *A. hydrophila*. External symptoms included pale body and gills, red hemorrhagic patches on the back, behind the operculum, fins, and other body parts, skin and muscle peeling with excessive mucus, frayed fins, and bloated or swollen abdomen. In severe cases, fungal infections and ulcers were observed.

Figure 1. External Anatomical Pathology of *Sangkuriang* Catfish (a) Healthy and normal fish (b) Diseased fish showing symptoms of frayed dorsal fins, hemorrhages, and ulcers at the base of the caudal fin

As shown in Figure 1, fish in this study exhibited hemorrhages at the base of the caudal and dorsal fins. Some fish also developed abdominal swelling after injection. These findings align with Yuasa et al. (2003), who reported that *A. hydrophila* infections generally cause widespread hemorrhagic septicemia, fin erosion, and abdominal swelling filled with fluid (dropsy), leading to mortality.

Survival rate

Based on observations of catfish over ten days following challenge testing with A. hydrophila, the average survival rate ranged from 83.33% to 100%. Variance analysis showed that different doses of pineapple peel flour added to the feed significantly affected the survival rate ($P<0.05$). Figure 2 indicates that all treatments with added pineapple peel flour had a higher survival rate than the control group without pineapple peel flour. Duncan's post hoc test showed that P0 was significantly different from P1, P2, and P3 ($P<0.05$).

According to Effendie (2002) in Anugraha et al. (2014), survival rate is influenced by internal and external factors. Internal factors originate from the fish itself; stress due to improper handling can lead to high mortality. External factors include pathogen infections and environmental changes. A. hydrophila infection in this study contributed to fish mortality, especially in treatments P0 and P1. Changes in clinical symptoms and anatomical pathology in infected catfish indicated abnormal conditions or disease, leading to mortality. The addition of 20-30% pineapple peel flour in the feed is believed to help enhance fish resistance to A. hydrophila infection by boosting immune responses.

Hematological parameters

The observed hematological parameters included hemoglobin, hematocrit, total erythrocytes, total leukocytes, and differential leukocytes. The hematological observations of Sangkuriang catfish maintained for 45 days are detailed in Table 1.

Table 1. Average hematological parameters and cellular immune response of catfish

Treatment	Day-	Average						
		Hemoglobin Level (g/dL)	Hematocrit Level (%)	Total Erythrocytes (x 10^6 cells/mm³)	Total Leukocytes (x 10^4 cells/mm³)	Lymphocytes (%)	Monocytes (%)	Neutrophils (%)
P0	0	3,20 ± 0,00[a]	16,33 ± 0,00[a]	0,91 ± 0,00[a]	3,18 ± 0,00[a]	89,00 ± 0,00[a]	7,00 ± 0,00[a]	4,00 ± 0,00[a]
	20	4,33 ± 0,12[b]	24,00 ± 2,36[d]	1,40 ± 0,18[b]	6,43 ± 0,59[a]	88,00 ± 1,00[a]	10,33 ± 0,58[b]	1,67 ± 0,58[a]
	37	6,73 ± 0,23[b]	26,05 ± 0,91[c]	2,34 ± 0,42[ab]	4,94 ± 0,18[a]	90,33 ± 1,15[b]	6,33 ± 0,58[a]	3,33 ± 0,58[ab]
	45	6,73 ± 0,31[a]	22,95 ± 0,87[a]	3,59 ± 0,26[a]	5,67 ± 1,34[a]	96,33 ± 0,58[b]	1,33 ± 0,58[a]	2,33 ± 0,71[a]
P1	0	3,20 ± 0,00[a]	16,33 ± 0,00[a]	0,91 ± 0,00[a]	3,18 ± 0,00[a]	89,00 ± 0,00[a]	7,00 ± 0,00[a]	4,00 ± 0,00[a]
	20	3,93 ± 0,70[a]	14,00 ± 1,01[b]	0,97 ± 0,12[a]	7,76 ± 0,64[a]	91,67 ± 1,53[c]	6,00 ± 1,00[a]	2,33 ± 0,58[a]
	37	5,67 ± 0,61[a]	7,95 ± 2,40[a]	2,81 ± 0,25[bc]	8,75 ± 0,30[b]	87,67 ± 1,53[a]	9,00 ± 1,00[b]	3,33 ± 0,58[ab]
	45	6,53 ± 1,67[a]	29,22 ± 1,64[b]	5,40 ± 0,63[b]	8,42 ± 0,46[b]	89,33 ± 1,53[a]	7,67 ± 2,08[b]	3,00 ± 1,00[a]
P2	0	3,20 ± 0,00[a]	16,33 ± 0,00[a]	0,91 ± 0,00[a]	3,18 ± 0,00[a]	89,00 ± 0,00[a]	7,00 ± 0,00[a]	4,00 ± 0,00[a]
	20	4,33 ± 0,58[a]	10,12 ± 2,34[a]	1,17 ± 0,12[ab]	6,58 ± 0,73[a]	91,00 ± 1,53[bc]	6,67 ± 1,15[a]	2,33 ± 0,58[a]
	37	4,93 ± 0,42[a]	14,53 ± 1,99[b]	3,37 ± 0,60[c]	9,13 ± 0,76[b]	88,00 ± 1,53[ab]	8,67 ± 1,15[b]	2,67 ± 0,58[a]
	45	5,80 ± 0,60[a]	22,97 ± 1,76[a]	3,04 ± 0,86[a]	8,63 ± 0,86[b]	89,67 ± 0,58[a]	7,00 ± 2,00[b]	3,33 ± 1,15[a]
P3	0	3,20 ± 0,00[a]	16,33 ± 0,00[a]	0,91 ± 0,00[a]	3,18 ± 0,00[a]	89,00 ±0,00[a]	7,00 ± 0,00[a]	4,00 ± 0,00[a]
	20	3, 67 ± 0,58[a]	18,97 ± 1,57[c]	1,06 ± 0,05[a]	6,90 ± 0,80[a]	91,33 ± 1,00[ab]	7,33 ± 0,58[a]	3,67 ± 0,58[a]
	37	5,27 ± 0,23[a]	15,88 ± 2,82[b]	2,01 ± 0,12[a]	9,49 ± 0,40[b]	88,00 ± 1,00[a]	7,67 ± 0,58[ab]	4,33 ± 0,58[b]
	45	6,00 ± 0,20[a]	32,16 ± 1,01[c]	2,64 ± 0,51[a]	6,17 ± 0,25[a]	91,33 ± 0,58[a]	6,00 ± 1,00[b]	2,67 ± 0,58[a]

a. Hemoglobin (Hb) levels

Observations showed that catfish hemoglobin levels ranged from 3.20±0.00 g% to 6.73±0.31 g%. Hemoglobin levels increased across all treatments by day 45. Variance analysis indicated that different doses of pineapple peel flour in the feed had no significant effect on hemoglobin levels (P>0.05). Table 1 shows that hemoglobin levels in treatments with pineapple peel flour were lower than in the control group.

Hemoglobin levels remained stable after *A. hydrophila* infection, suggesting that pineapple peel flour helped protect the fish from infection, as hemoglobin levels remained within a normal range. Muliani (2017) stated that increased hemoglobin levels are attributed to flavonoids and tannins, which act as antioxidants and protect hemoglobin from oxidation.

b. Hematocrit (He) levels

Hematocrit levels ranged from 7.95±2.40% to 32.16±1.01%. Table 1 shows that the highest hematocrit percentage was in P3 and the lowest in P1. Hematocrit levels generally increased from day 0 to day 45. Variance analysis showed that different doses of pineapple peel flour significantly affected hematocrit levels (P<0.05). Duncan's post hoc test on hematocrit levels at day 45 showed that P0 was significantly different from P1 and P3 but not from P2. P1 was significantly different from P0, P2, and P3, while P3 was significantly different from P0, P1, and P3.

The increase in hematocrit levels is believed to result from the bioactive compounds in pineapple peel flour, which enhance hematocrit levels in fish blood. This aligns with Abdullah (2008), who reported that hematocrit values decrease in catfish (*Clarias sp.*) infected with *A. hydrophila*.

Hematocrit levels influence red blood cell volume, with normal catfish (*Clarias sp.*) hematocrit values ranging from 30.8% to 45.5% (Dopongtonung, 2008). In this study, hematocrit percentages were below normal, but the fish remained healthy as no mortality occurred during the study. The relatively small fish size may explain the lower hematocrit levels.

c. Total erythrocytes (red blood cells)

The total erythrocyte count ranged from $0.91 \pm 0.00 \times 10^6$ to $5.40 \pm 0.63 \times 10^6$ cells/mm³. Table 1 shows that the highest erythrocyte count was in P1 at day 45, while the lowest was observed in all treatments at day 0. Erythrocyte levels generally increased from day 0 to day 45. Variance analysis indicated that different doses of pineapple peel flour significantly affected total erythrocytes ($P<0.05$). Duncan's post hoc test at day 45 showed that P3 was significantly different from P1 but not from P0 and P2.

The highest total erythrocyte count was observed in P1 (10% pineapple peel flour). The increase in erythrocytes is likely due to the fish returning to normal physiological conditions. Fish produce new blood cells to replace those damaged by *A. hydrophila* infection. The flavonoid content in pineapple peel is thought to contribute to this increase. Hakim et al. (2016) stated that flavonoids have anti-inflammatory properties, helping to repair damaged tissue and improve blood-producing organ function.

According to Bangsa et al. (2015), stress affects fish health by impairing blood cell function. Additionally, inadequate nutrient supply to cells, tissues, and organs can affect erythrocyte production. Table 1 shows that erythrocyte levels remained within normal ranges during the study. Alamanda et al. (2007) and Lukistyowati & Windarti (2007) reported that normal fish erythrocyte counts range from $1\text{-}3 \times 10^6$ cells/mm³, while Burhanuddin (2014) stated that normal erythrocyte counts range from 20,000 to 3,000,000 cells/mm³. Based on these findings, the erythrocyte levels in *Sangkuriang* catfish in this study indicate overall healthy and normal fish.

d. Total leukocytes (white blood cells)

Based on observations in this study, the total leukocyte count in catfish had an average range of $3.18 \pm 0.00 \times 10^4$ - $9.49 \pm 0.40 \times 10^4$ cells/mm³. According to Table 1, after the challenge test, the highest total leukocyte count was found in P3, followed by P2, then P1, with the lowest result in P0. In general, there was an increase in total leukocytes in all treatments on day 37, following the challenge test, but a decrease was observed on day 45, which was the recovery phase.

Variance analysis showed that the addition of different doses of pineapple peel powder in feed had a significant effect on total leukocyte count in Sangkuriang catfish ($P < 0.05$). Duncan's multiple range test on total leukocytes on day 37 showed that P0 was significantly different from P1, P2, and P3. Meanwhile, on day 45, P0 was significantly different from P1 and P2 but not significantly different from P3. P1 was significantly different from P0 and P3 but not significantly different from P2.

The findings indicate that P0 catfish were not given the addition of pineapple peel powder, meaning they relied solely on their natural immunity, which resulted in a slower recovery process (Ratnasari et al., 2020). An increase in leukocyte count indicates the body's response to pathogens, as infected fish produce more leukocytes to phagocytose bacteria and synthesize antibodies (Mahasri et al., 2011). Leukocyte levels in fish infected with pathogens will increase as a defense mechanism (Martins et al., 2008). Consequently, the total leukocyte count increased after infection with A. hydrophila, indicating that the fish experienced bacterial infection (Purnamasari et al., 2015). The leukocyte counts observed in this study were still within the normal range, as stated by Lestari et al. (2012) and Noercholis et al. (2013), who reported that normal leukocyte levels range between 20,000-150,000 cells/mm³.

e. Differential leukocyte count

Lymphocyte percentage ranged from $87.67 \pm 1.53\%$ - $96.33 \pm 1.53\%$. Monocyte percentage ranged from $1.33 \pm 0.58\%$ - $10.33 \pm 0.58\%$. Neutrophil percentage ranged from $1.67 \pm 0.58\%$ - $4.33 \pm 0.58\%$. Variance analysis showed that the addition of different doses of pineapple peel powder in feed had a significant effect on the lymphocyte percentage in Sangkuriang catfish ($P < 0.05$). Duncan's test on lymphocyte levels indicated that P0 was significantly different from P1, P2, and P3. In P1, P2, and P3 (fish given pineapple peel powder), lymphocyte percentages increased from day 0 to day 20 but decreased on day 37, likely due to an increase in immune response after feeding.

This finding aligns with Ginting et al. (2021), who stated that an increase in lymphocyte percentage is a sign of a successful immune system response (non-specific immune response). According to Rustikawati (2012), lymphocyte levels decrease because many lymphocytes migrate to other circulatory spaces, competing to enter inflamed tissues. From Table 1, the monocyte percentage in this study ranged from 2.00±1.73% - 10.00±1.00%. Variance analysis showed that different doses of pineapple peel powder in feed significantly affected monocyte percentages in Sangkuriang catfish ($P < 0.05$). Duncan's test on monocyte levels on day 45 indicated that P0 was significantly different from P1, P2, and P3.

Monocyte percentages in catfish fed with pineapple peel powder decreased from day 0 to day 20. A steady decline in monocyte levels relates to their role as macrophages—since there was no infection at this stage, monocytes were not required for phagocytosis (Rahma et al., 2015). However, after the A. hydrophila challenge test, monocyte percentages increased, suggesting that the immune system had responded. The prebiotics in pineapple peel helped the fish detect and react to foreign pathogens. Neutrophil percentages ranged from 1.67±0.58% - 4.33±0.58%. Variance analysis showed that different doses of pineapple peel powder significantly affected neutrophil percentages ($P < 0.05$). Duncan's test on neutrophil levels on day 37 revealed that P2 was significantly different from P3 but not significantly different from P0 and P1. Generally, neutrophil counts increase during infections because neutrophils migrate from the bloodstream to infected tissues (Rahma et al., 2015). However, the decrease in neutrophil percentages corresponded to an increase in lymphocyte percentages in fish fed with pineapple peel powder. This decline in neutrophils occurred due to autolysis—the breakdown of neutrophils after they successfully fought off microbial infections (Rustikawati, 2012).

Water quality parameters

Several water quality parameters observed in this study include temperature, pH, dissolved oxygen, and ammonia. The results of water quality measurements during the 45-day maintenance period of Sangkuriang catfish are shown in Table 2 below.

Table 2. Range of Water Quality Parameters During the Study

Parameter	Treatment	Optimal Range
	P0	P1
Temperature (°C)	28.7 - 31.2	28.7 - 31.2
DO (mg/L)	2.8 - 5.6	3.1 - 5.7
pH	7.17 - 8.18	7.24 - 8.28
Ammonia (mg/L)	0.009 - 0.474	0.009 - 0.282

During the 45-day maintenance period, the recorded water temperature ranged between 28.7 - 31.2°C. The optimal temperature for Sangkuriang catfish farming is 24 - 30°C (Djoko, 2006). Temperature is a crucial water quality parameter in aquaculture, as an optimal temperature ensures efficient metabolism in fish, which positively impacts their growth and survival. High temperatures can reduce dissolved oxygen levels in water (Mulyani and Johan, 2020).

The dissolved oxygen (DO) levels during the maintenance period ranged from 2.8 - 7.6 mg/L, which is still considered optimal for Sangkuriang catfish. Dissolved oxygen for catfish should not be lower than 3 mg/L (Laheng and Widyastuti, 2019). According to Ratnasari (2020), oxygen levels exceeding 3 mg/L are ideal for optimal catfish growth.

The pH levels during the study ranged between 7.01 - 8.50, which is still within the optimal range for Sangkuriang catfish farming. According to Purwanti and Sudaryono (2014), catfish can thrive in a pH range of 6.5 - 9. Effendi (2003) stated that most fish can adapt well in aquatic environments with a pH range of 5 - 9. pH is an essential factor in determining the acidity or alkalinity of water. Maintaining a stable and optimal pH level is crucial for successful catfish farming.

The ammonia (NH_3) levels during the maintenance period ranged from 0.009 - 0.474 mg/L. According to Pilay (2004), toxic ammonia levels range from 0.6 - 2.0 mg/L, indicating that the ammonia levels observed in this study were within the safe range. Ammonia is a compound produced from protein catabolism and excreted by organisms, resulting from the decomposition of organic matter by bacteria. This study suggests that adding pineapple peel flour to fish feed at different doses did not deteriorate water quality. This is likely because all the feed was fully consumed, preventing leftover feed from contaminating the water and keeping it clean.

CONCLUSION

Based on the research findings on the potential of pineapple peel flour (*A. comosus*) in controlling A. hydrophila bacterial infection in Sangkuriang catfish (*C. gariepinus*), the following conclusions can be drawn:

1. The addition of pineapple peel flour at different doses in the feed did not have a significant effect on the clinical symptoms of Sangkuriang catfish after infection with *A. hydrophila*, as all treatments exhibited similar clinical signs. However, it significantly affected the anatomical pathology of the fish, as no mortality was observed in treatments where pineapple peel flour was added.
2. The addition of pineapple peel flour at different doses in the feed had a significant effect ($P<0.05$) on the survival rate of Sangkuriang catfish after infection with *A. hydrophila*.
3. The addition of pineapple peel flour at different doses in the feed had a significant effect ($P<0.05$) on hematocrit levels, total erythrocytes, total leukocytes, and the percentage of lymphocytes, monocytes, and neutrophils in Sangkuriang catfish after infection with *A. hydrophila*.
4. The optimal dose of pineapple peel flour in the feed for controlling *A. hydrophila* infection was 20%.

REFERENCES

Abdullah, Y. 2008. Efektivitas ekstrak daun paci-paci (*Leucas lavandulaefolia*) untuk pencegahan dan pengobatan infeksi penyakit MAS (*Motile Aeromonas Septicaemia*) ditinjau dari patologi makro dan hematologi ikan lele dumbo (*Clarias* sp.). Skripsi Fakultas Perikanan dan Ilmu Kelautan. IPB: Bogor.

Alamanda, E.L, N.S. Handajani, dan A. Budiharjo. 2007. Penggunaan metode hematologi dan pengamatan endoparasit darah untuk penetapan kesehatan ikan lele dumbo (*Clarias gariepinus*) di kolam budidaya desa Mangkubumen Boyolali, Biodiversitas, 8 (1) 34-38.

Anugraha, R. S., dan E. Arini. 2014. Pengaruh penggunaan ekstrak buah nanas terhadap tingkat pemanfaatan protein pakan dan pertumbuhan ikan mas (*Cyprinus carpio*). Journal of Aquaculture Management and Technology, 3(4), 238-246

Bangsa, P.C., Sugito., Zuhrawati., R. Daud, N. Asmilia, dan Azhar. 2015. Pengaruh peningkatan suhu terhadap jumlah eritrosit ikan nila (*Oreochromis niloticus*). Jurnal Medika Veteriner. 9 (1) 9-11.

Burhanuddin, A.I. 2014. Ikhtiologi ikan dan segala aspek kehidupannya. Deepublish, Yogyakarta.

Djoko, 2006. Lele sangkuriang alternative kualitas ditanah priang. Jakarta

Dopongtonung, A. 2008. Gambaran darah ikan lele (*Clarias* sp.) yang berasal dari daerah Laladon Bogor. Skripsi. Fakultas Kedokteran Hewan Institut Pertanian Bogor.

Effendi, H. 2003. Telaah kualitas air bagi pengelolaan sumberdaya dan lingkungan perairan. Cetakan Kelima. Yogyakarta, Kanisius. 87 hal.

Effendie, M.I. 2002. Metode Biologi Perikanan. Bogor: Yayasan Pustaka Nusantara.

Ginting, K. D., M. Riauwaty, dan H. Syawal. 2021. Diferensiasi leukosit ikan lele dumbo (*Clarias gariepinus*) yang diberi pakan mengandung kunyit (Curcuma domestica val.) dan diinfeksi bakteri Aeromonas *hydrophila.* Jurnal Ilmu Perairan (Aquatic Science), 9(2), 116-125.

Hakim, R.F., F. Fakhrurrazi, dan W. Ferisa. 2016. Pengaruh air rebusan daun salam (*Eugenia polyantha* w.) Terhadap pertumbuhan *Enterococcus faecalis*. Journal Dentistry Society, 1(1):21-28.

Laheng, S. dan A. Widyastuti. 2019. Pengaruh lama perendaman menggunakan air kelapa terhadap maskulisasi ikan lele masamo (*Clarias* sp). Acta Aquat. 6 :(2) 58– 63.

Lestari, E., T.R. Setyawati, dan A.H. Yanti. 2017. Profil hematologi ikan gabus (*Channa striata* Bloch, 1793). J. Protobiont. 6 :(3) 283–289.

Lukistyowati, I., Windarti, dan M. Riauwaty. 2007. Studi hematologi ikan-ikan yang dipelihara di Kotamadya Pekanbaru. Laporan Hasil Penelitian Lembaga Penelitian Universitas Riau. 50 Hlm.

Mahasri, D., P. Widyastuti, dan L. Sulmatiwi. 2011. Gambaran leukosit darah ikan koi (*Cyprinus carpio*) yang terinfestasi *Ichthyophthirius multifiliis* pada derajat infestasi yang berbeda dengan metode kohabitasi. Jurnal Ilmiah Perikanan dan Kelautan, 3(1).

Martins, Ml., J. Mourino, G. Amaral, F. Vieira, G. Dotta, A. Jatoba, F. Pedrotti, G. Jeronimo, N. Buglione, dan G. Pereira. 2008. Haematological changes in Nile tilapia experimentally infected with *Enterococcus* sp. Brazilian Journal of Biology, 68(3), 657-661.

Mulia, D.S. 2010. Isolasi, Karakterisasi, dan Identifikasi Bakteri *Aeromonas* sp. Penyebab Penvakit *Motile Aeromonas Septicemia* (MAS) pada Gurami. Jurnal Sains Akuatik 13 (2): 9-16.

Muliani. 2017. Pengaruh pemberian simplisia daun salam *Syzygium polyantha* walp. terhadap eritrosit, hemoglobin, pcv, dan leukosit broiler yang menderita cekaman panas. Skripsi. Fakultas Kedokteran. Universitas Hasanuddin. 59 hlm.

Mulyani, H.S., dan T.I. Johan. 2020. Pengaruh ekstrak daun kersen (*Muntingia calabura*) dengan dosis berbeda terhadap lama inkubasi, daya tetas dan kelulushidupan larva ikan lele dumbo (Clarias gariepinus). Jurnal Dinas Pertanian XXXVI :(1) 99–110.

Muslikha, M., S. Pujiyanto, S.N. Jannah, dan H. Novita. 2016. Isolasi karakterisasi *Aeromonas hydrophila* dan deteksi gen penyebab penyakit MAS *(Motile Aeromonas Septicemia*) dengan 16s rrna dan aerolysin pada ikan lele (*Clarias* sp.). Jurnal Akademika Biologi, *5*(4), 1-7.

Noercholis, A., dan M. Maftuch. 2013. Ekstraksi fitur roundness untuk menghitung jumlah leukosit dalam citra sel darah ikan. J. EECCIS. 7 :(1) 35–40.

Nurhayati. 2013. Penampilan ayam pedaging yang mengkonsumsi pakan mengandung kulit nanas disuplementasi dengan yoghurt. Jurnal Agripet 13(2) :15-20.

Pillay T.V.R. 2004. Aquaculture and the environment. Second Edition. UK: Blackwell Publishing.

Purnamasari, L., A.D. Sasanti, dan Yulisman. 2015. Perendaman ikan lele sangkuriang (*Clarias* sp.) dalam sari buah belimbing wuluh untuk mengobati infeksi *Aeromonas hydrophila*. Jurnal Akuakulutur Raw Indonesia. 82-93

Purwanti, S. C., dan A. Sudaryono. 2014. Gambaran profil darah ikan lele sangkuriang (*Clarias gariepinus*) yang diberi pakan dengan kombinasi pakan buatan dan cacing tanah (*Lumbricus rubellus*). Journal of Aquaculture Management and Technology, 3(2), 53–60.

Rahma, F.W., G. Mahasri, dan L. Surmartiwi. 2015. Pengaruh pemberian ekstrak *Sargassum* sp. dengan pelarut metanol pada pakan terhadap jumlah eritrosit dan differensial leukosit ikan lele dumbo (*Clarias gariepinus*). Fakultas Perikanan dan Kelautan Universitas Airlangga Jurnal Ilmiah Perikanan dan Kelautan. Vol. 7: 2.

Raihan, 2023. Kenali ragam penyakit ikan lele: gejala dan solusinya. https://digitani.ipb.ac.id/4250-2/ Diakses pada tanggal 13 Maret 2024

Ratnasari, D. 2011. Teknik pembesaran ikan lele dumbo (*Clarias gariepinus*) Di Biotech Agro, Kabupaten Jombang, Propinsi Jawa Timur. Skripsi. Universitas Airlangga, Surabaya.

Rustikawati, I. 2012. Efektivitas Ekstrak *Sargassum* sp. terhadap diferensiasi leukosit ikan nila (*Oreochromis niloticus*) yang diinfeksi *Streptococcus iniae*. Jurnal Akuatika 3(2) :125-134.

Saptiani, G. 2009. Daya fagositik pertahanan selluler pada ikan lele *(Clarias batrachus)* yang ditantang dengan bakteri *Streptococcus* sp. Prosiding Seminar Nasional Perikanan 2009, Jakarta, 3-4 Desember. 92-101.

Sukri, S.A.M., Yusrina., T.H. Zuharlida., S. Shazani., F.P. Naim., S.W. M. Lee M., A.O. Dawood., A.K. Zulhisyam. 2022. Effect of feeding pineapple waste on growth performance, texture quality and flesh colour of nile tilapia (*Oreochromis niloticus*) fingerlings. Saudi Journal of Biological Sciences Volume 29, Issue 4, Pages 2514-2519.

Sumiati, T., M. Eem, dan M. Intan. 2020. Potensi ekstrak bonggol nanas (*Ananas comosus* (L.) Merr.) sebagai obat kumur. Jurnal Katalisator, 5(2), 215-223

Triyaningsih., Sarjito, S.B. Prayitno. 2014. Patogenisitas *Aeromonas hydrophila* yang diisolasi dari lele dumbo (*Clarias gariepinus*) yang berasal dari Boyolali. Journal of Aquaculture Management and Technology. 3(2): 11- 17.

Van Doan, H., C. Lumsangkul, S.H. Hoseinifar, S. Tongsiri, C. Chitmanat, M.S. Musthafa, E. El-Haroun, E. Ringo. 2021. Modulation of growth, innate immunity, and disease resistance of Nile tilapia (*Oreochromis niloticus*) culture under biofloc system by supplementing pineapple peel powder and *Lactobacillus plantarum*. Fish Shellfish Immunol. 155, 212-220.

Widyawati, R., N. Dhia, dan W. Dyah. 2020. Ekstrak buah nanas terhadap jumlah total leukosit dan neutrofil ikan lele (*Clarias batrachus*) yang diinfeksi dengan *Aeromonas hydrophila*. Universitas Wijaya Kusuma Surabaya: Jurnal Vitek Bidang Kedokteran Hewan Vol.10.