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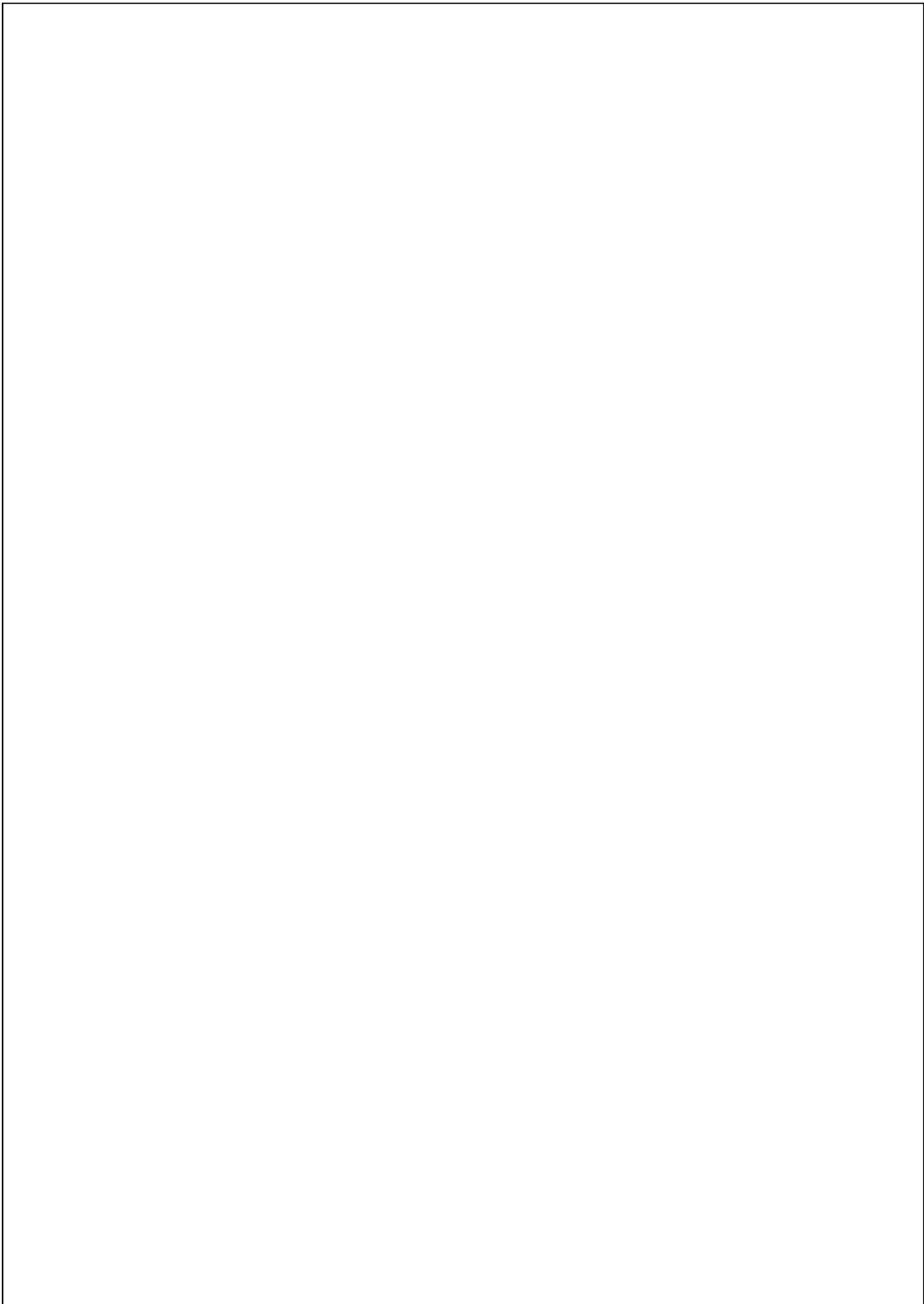
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Experimental infection of *Streptococcus agalactiae* in silver rasbora (*Rasbora argyrotania*): Effect to hematological profile from infected fish

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Abstract. Silver rasbora (*Rasbora argyrotania*) is one of Indonesian local freshwater fishes species that have high economic value. One of bacterial diseases that probably infected to silver rasbora cultivation is *Streptococcus agalactiae* that caused streptococcosis. There was limited information about effect of *Streptococcus agalactiae* infection to hematological profile in silver rasbora has never been reported before. The aims of these studies were to determine the hematological profile of silver rasbora that experimentally infection with *Streptococcus agalactiae*. A total of four hundreds silver rasbora (6 ± 0.1 cm length and 3.6 ± 0.2 grams weight) were intramuscularly infection with 0,1 mL *Streptococcus agalactiae* in different density, include 10^4 , 10^6 , 10^8 , 10^{10} and NaCl physiological solution as negative control. Observation of hematological profile was carried out by blood examination post infection which included total erythrocytes, total leukocytes, differential leukocytes (percentage of lymphocyte, neutrophil, and monocyte) and hemoglobin levels. Hematological profile in silver rasbora (*Rasbora argyrotania*) infected by *Streptococcus agalactiae* showed an increase in neutrophil and monocyte percentage and showed a decrease in total erythrocytes, hemoglobin, total leukocytes and lymphocyte percentage. Further studies were needed to prevent the *Streptococcus agalactiae* infection using herbal medicine.

1. Introduction

Silver rasbora is one of Indonesia's local freshwater fish species, that have high economic value and now being cultivated [1]. An aquaculture business cannot be separated from problems or obstacles in the cultivation process. Constraints in the process of fish culture can be caused by the presence of *S. agalactiae* bacterial disease which can cause Streptococcosis [2].

The first case of the attack of *S. agalactiae* was reported by Hoshina *et al.* [3] that infected *Rainbow trout* in Japan in 1957 and became the most common infection in freshwater fish culture. Streptococcosis can cause high mortality rates of more than 50% for 3 to 7 days after infection by showing clinical symptoms of irregular swimming fish (whirling), the body forming the letter 'C', changes in body color, and increasing of opening and closing operculum [4].

Chideroli *et al.* [5] stated that cases of Streptococcosis that infected tilapia showed mortality rates ranging from 56.67% to 100% in tilapia culture in Brazil. Bowater *et al.* [6] also stated that the infection of *S. agalactiae* occurred in 2007 and 2011 in Australia can infect groupers by showing clinical symptoms of exophthalmia, haemorrhagic, lethargy, and inflammation.



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The ability of *S. agalactiae* to infect fish is due to the presence of dissolved toxins in extracellular product (ECP). The toxin produced in the form of hemolysin/cytolysin and CAMP is a pathogenic factor in fish that can cause meningoencephalitis which is characterized by thickening of dense blood vessels and inflammatory infiltration cells and the occurrence of septicemia in fish [7].

According to Alsaid *et al.* [8] blood examination (hematology) can be used as an indicator to diagnose a disease and determine fish health. This study aims to analyze the hematological profile of silver rasbora after being infected by *S. agalactiae*.

2. Material and method

This study was conducted in the Laboratory of the Universitas Airlangga Campus Banyuwangi.

2.1. Experimental Fish Preparation

Silver rasbora measuring 6 ± 0.1 cm are 20 fish each aquarium. The experimental fish was immersed in a 30 ppm salt solution for 15 minutes and acclimatized for 7 days in an aquarium before infection.

2.2. *Streptococcus agalactiae* Preparation

The *S. agalactiae* isolate was recultured on B₅B (Brain Heart Infusion Broth) media then incubated for 24 hours at 37°C and recultured on BHIA (Brain Heart Infusion Agar) media, incubated at 37°C for 24 hours. Identification of bacteria using Gram staining, motility test using Sulfide Indole Motility (SIM) media, oxidative-fermentative test using OF (oxidative-fermentative) media and catalase test using hydrogen peroxide (H₂O₂) solution. The biochemical test results were equated with the characteristics of the *S. agalactiae* refers to Macfaddin [9], Barrow and Feltham [10], and Holt [11].

2.3. Infection of *S. agalactiae* to Silver Rasbora

The infection of *S. agalactiae* to silver rasbora consist of four treatments (injection 0,1 ml of 10⁴ CFU/ml, 10⁶ CFU/ml, 10⁸ CFU/ml, and 10¹⁰ CFU/ml *S. agalactiae*) and one negative control (injection 0,1 of NaCl physiologic) with quadruplicate. The observations of behavioral changes, clinical symptoms, and hourly mortality rates for 28 hours.

2.4. Rearing of Experimental Fish

Silver rasbora were reared for 2 days after infection. Feeding is done twice a day (morning and evening) with ad satiation method. Temperature and pH measurements were taken at the time of feeding while DO and ammonia measurements were taken at the beginning and end of maintenance. Substitution of water is done by siphoning every morning as much as 50% from the total volume.

2.5. Fish Blood Sampling

Fish blood sampling at the caudal artery with a 1 ml syringe with anticoagulant (EDTA). Each blood sample taken from each treatment was inserted into a microtube to analyze the blood profiles. Observation of blood profile was carried out by hematological examination which included total erythrocytes [12], total leukocytes [12], differential leukocytes [12], and hemoglobin levels [13].

3. Result and discussion

3.1 Result

The results of the hematological profile of silver rasbora after infection with *S. agalactiae* infection were presented in **Table 1** and data on water quality during study were presented in **Table 2**.

Table 1. Hematology Profile of Silver rasbora after *S. agalactiae* Infection

Observed Parameter	Treatment					Normal Value of Cyprinidae
	P0	P1	P2	P3	P4	
Erythrocytes (x 10 ⁶ sel/ml)	1.2±0.006	0.8±0.031	0.7±0.025	0.5±0.021	0.2±0.009	1.05-3.0 [14]
Leukocytes (x 10 ⁴ sel/ml)	11.8±0.191	11.5±0.004	11.2±0.004	10.5±0.202	9.1±0.085	9.7-11.63 [15]
Hemoglobin (g%)	7.9±0.1	7.3±0.1	6.2±0.2	4.6±0.2	4.5±0.1	4.5-7.06 [16]
Neutrophils (%)	28±1	29.5±0.5	32.5±1.5	33±0	33.6±0.5	33.6-39.1 [4]
Monocyte (%)	29.5±0.5	30±0	32.5±0.5	34.5±0.5	37±0	29.1-30.8 [4]
Lymphocytes (%)	42.5±0.5	40.5±0.5	35±1	32.5±0.5	29.5±0.5	31.4-33.2 [7]

Note : P0 (control), P1 = injection 104 CFU/ml, P2 = injection 106 CFU/ml, P3 = injection 108 CFU/ml, P4 = injection 1010 CFU/ml.

Tabel 2. Water Quality Measurement Results for Silver rasbora during study.

Parameter	Results
Temperature (°C)	27 – 28
pH	6 – 7
DO (ppm)	6 – 8
Ammonia (ppm)	0,1

3.2 Discussion

S. agalactiae infection in silver rasbora can cause changes in hematological profile and blood glucose levels. According to Pavlidis *et al.* [17] the health status of fish can be seen through a hematological profile that includes the number of erythrocytes, the number of leukocytes, the percentage of hemoglobin and differential leukocytes.

Based on the results, the total of erythrocytes before the infection with *S. agalactiae* showed a value of $1.2 \pm 0.006 \times 10^6$ cells / ml. These results indicate the same value as the normal erythrocyte count in Cyprinidae ranging from $1.05-3.0 \times 10^6$ cells / ml [14]. The number of erythrocytes after infection by *S. agalactiae* showed lower than normal values in all treatments ranging from 0.2 ± 0.009 to $0.8 \pm 0.031 \times 10^6$ cells / ml, except for the negative control (P0), $1.2 \pm 0.006 \times 10^6$ cells / ml. Celik and Birkan [18] said that the decrease of the number of erythrocytes was caused by anemia in fish which was marked by bleeding. Its caused by *S. agalactiae* can produce hemolysin / cytolysin toxin which is able to lyse erythrocyte cells and impact to the decreasing of the value of erythrocytes lower than the average value of normal erythrocyte in Cyprinidae fish.

The concentration of hemoglobin (Hb) in silver rasbora ranged from $4.5 \pm 0.1-7.3 \pm 0.1$. The concentration of normal hemoglobin values in Cyprinidae fish ranged from $4.5-7.06$ g% [16]. The low amount of haemoglobin in infected fish occurred by the bleeding in fish, low concentration of hemoglobin indicates anemia in the fish's body [19]. Hardi *et al.* [20] reported that hemoglobin levels in the blood were related to blood plasma osmolarity balance, it was suspected that *S. agalactiae* secreted hemolysin / cytolysin toxins that affect the stability of hemoglobin. The toxin can cause decreased blood plasma osmolarity and erythrocyte lysis which causes decreasing Hb levels and resulting low energy.

The calculation of total leukocytes ranged from 9.1 ± 0.085 to $11.5 \pm 0.004 \times 10^4$ cells / ml. According to Moyle and Cech [15] the number of normal leukocytes in Cyprinidae fish ranges from 9.7 to 11.63×10^4 cells / ml. Hardi *et al.* [20] stated that *S. agalactiae* infection causes the increasing of leukocyte cells in the infected area as a defense effort. Leukocyte cells work as phagocyte cells to bacteria in the blood so the pathogen cannot develop and spread the virulence factors to the host.

Percentage of neutrophil in all treatments showed normal range between 28 ± 1 and $33.6 \pm 0.5\%$. According to Tripathi *et al.* [21] the number of neutrophils in the blood of Cyprinidae fish ranged from 31.4 to 33.26% . An increase in the number of neutrophils indicates that the fish's body has formed the body's defense system. When bacterial infection occurred, neutrophils are produced by lymph migrated

to the site of infection, therefore the number of neutrophils usually increases. While the decrease in the percentage of neutrophil cells is caused by neutrophil cells working to eliminate pathogens that enter the tissue in the fish's body [22].

The total percentage of monocytes in all treatments ranged from 29.5 ± 0.5 - $37 \pm 0\%$. Rashidi et al. [4] stated that the percentage of normal monocytes in Cyprinidae fish was 29.12-30.81%. The observations showed the highest monocyte value after *S. agalactiae* infection in P4 treatment (injection of *S. agalactiae* 1010 CFU / ml). The increase in the percentage of monocytes that are still in the normal range indicates the response of leukocytes to foreign bodies or disease agents found in the body to play an active role in phagocyte-causing agents [23].

The percentage of lymphocytes in all treatments ranged from 29.5 ± 0.5 to $42.5 \pm 0.5\%$. The number of normal range lymphocytes in Cyprinidae fish according to Neelima et al. [7] ranged from 31.4 to 33.26%. According to Uribe et al. [23] stated that lymphocytes are concentrated in the anterior tissue of the kidney and spleen. After lymphocyte infection will move to the tissue and fight pathogens.

Water quality parameters (Tabel 2.) includes temperature, pH, DO, and ammonia showed normal range for maintaining silver rasbora [24]. The temperature during the maintenance of silver rasbora ranges from 27-28 °C. According to Rosadi et al. [24] silver rasbora live at an average temperature of 25.5-31.6 °C. The pH of water during the study ranged from 6-7. According to Baensch and Riehl [25] silver rasbora live at pH 6.5-7. The results of dissolved oxygen (DO) measurements during the study ranged from 6-8 mg / L. According to Rosadi et al. [24] the dissolved oxygen content (DO) for silver rasbora ranged from 4.2-7.5 mg / L. The results of the ammonia content during the study were 0.1 mg / L. This is consistent with the statement of Boyd and Lichtkoppler [26] that the ammonia concentration that is safe for the organism is <1 mg / L.

4. Conclusion

The conclusion on this study were hematological profile in silver rasbora (*Rasbora argyrotaenia*) infected by *Streptococcus agalactiae* showed an increase in neutrophil and monocyte percentage and showed a decrease in total erythrocytes, hemoglobin, total leukocytes and lymphocyte percentage. Further studies were needed to prevent the *Streptococcus agalactiae* infection using herbal medicine.

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