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HEMATOLOGY PARAMETERS OF NILEM PADJADJARAN STRAIN (*Osteochilus* sp.) INFECTED by *Aeromonas hydrophila*

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KeyWords
Aeromonas hydrophila, hematology parameter, nilem padjadjaran strain, white blood cells, red blood cells, hematocrit

ABSTRACT

This study aims to determine the description of the blood of nilem padjadjaran strain against the *Aeromonas hydrophila* infection. The research was conducted on February 2 - March 31, 2018 at Aquaculture Laboratory and Laboratory of Microbiology and Molecular Biotechnology Faculty of Fishery and Marine Sciences, Universitas Padjadjaran. The treatment are common carp, nilem and nilem padjadjaran strain infected by *A. hydrophila* with 10^8cfu/ml intraperitoneally. Parameters observed included white blood cells, red blood cells, hematocrit and water quality. The number of white blood cells and red blood cells of nilem padjadjaran strain not tend to differ significantly with common carp as its male parent. The number of white blood cells of common carp, nilem and nilem padjadjaran strain before the challenge test is 63.467, 71.733 and 64.800 cells/mm^3, but after the tenth hour challenge becomes 124.267, 126.133, 119.733 cells/mm^3. The number of red blood cells of common carp, nilem and nilem padjadjaran strain before the challenge test is 2.133.333, 2.466.667 and 2.126.667 cells/mm^3, after the tenth hour challenge becomes 1.096.667, 1.410.000 and 1.096.667 cells/mm^3. Hematocrit common carp, nilem and nilem padjadjaran strain before the challenge test is 31%, 33% and 31%, while hematocrit post-challenge test turned into 19%, 20% and 20%.

INTRODUCTION

Nilem padjadjaran strain (*Osteochilus* sp.) is one of the new freshwater fish species that emerged in the community. The fish is the result of cross-breeding between female nilem (*Osteochilus hasselti*) and male common carp (*Cyprinus carpio*). This cross yields the dominant female breed and the morphology leads to the nilem fish. The number of female gender percentage of nilem padjadjaran strain fish compared to normal fish nilem become its own advantages. The larger proportion of nilem padjadjaran strain female fish serves as the carrying capacity to maximize the production of nilem fish eggs as caviar raw material or for spawning (Abidin 2013). In addition, according to Mulyadi et al. (2017) hybrid nilem fish has faster growth than ordinary nilem, this is because of the growth gene inheritance of common carp into hybrid nilem body. Based on the Abidin (2013) study, at the same age, the nilem padjadjaran fish has a total length of 64.12 ± 4.54 mm, while the normal nilem fish is 46.48 ± 5.36 mm. Nilem padjadjaran strain weight is certainly superior compare to normal nilem, which is 3.39 ± 0.70 grams while normal nilem is 1.32 ± 0.41 grams. Hybridization is also performed to obtain derivatives with higher environmental and disease resistance, more attractive colors and shapes for ornamental fish and better meat quality (Arianto and Utami 2006).

Increased demand for nilem fish in the market, encouraging the development of technology cultivation with intensive system. But in practice, intensive cultivation often experience various problems such as the emergence of disease attacks. Such disease attacks can lead to crop failure and economic losses.

Fish diseases are divided into two types, namely infectious and noninfectious diseases. Infectious disease is a disease caused by several groups of animals, namely parasites, bacteria and viruses. Noninfectious diseases are caused by unsuitable environmental factors, poor nutrition and genetic factors (Rosidah 2016). One of the bacterial diseases that attack the nilem fish is a bacterial disease that also commonly attacks other freshwater fish species, namely *Aeromonas hydrophila*.

A. hydrophila is a bacteria that is oxidative, anaerobic facultative, can ferment sugars and do not form spores, also is a native inhabitant of the aquatic environment. These bacteria spread rapidly in fish with high stocking densities and can result in seed deaths of up to 90%. The disease caused by *A. hydrophila* is opportunistic, which is able to develop more malignant in a bad environment and stressful fish (Rosidah 2016).

A. hydrophila as the cause of Motile Aeromonas Septicemia (MAS) disease is very influential in freshwater fish cultivation and often cause disease outbreaks with high mortality rate (80-100%) and in a relatively short time, ie 1-2 weeks (Triyaningsih et al. 2014). Infected fish usually have bruises on their body, but can also show other signs, such as protrusion of the eyeball (exophthalmia), bleeding in some parts of the body and abdomen swell. According to Kordi (2004), transmission of *A. hydrophila* bacteria can take place through contaminated cultivation equipment. In addition, according to Manurung and Susantie (2017), transmission of *A. hydrophila* bacteria can take place through water, body contact and contact with contaminated equipment.

Diseases infection in nilem padjadjaran strain can cause decreased body resistance. This decrease in fish endurance can be seen through changes in hematology (blood conception). Therefore, this research was conducted by using nilem padjadjaran strain as the material tested to know the hematology parameters of nilem padjadjaran strain fish in the form of white blood cells, red blood cells and hematocrit compared with common carp and nilem by conducting a challenge test using *A. hydrophila*.

METHOD OF RESEARCH

The research was conducted at Aquaculture Laboratory and Molecular Biotechnology Laboratory of Fishery and Marine Science Faculty of Padjadjaran University on February 2 - March 31, 2018. This research used experimental method with Completely Randomized Design with three treatments and three replications. Treatment used was the maintenance of common carp, nilem and nilem padjadjaran strain that infected by *A. hydrophila* 10^8cfu/ml intraperitoneally. Each aquarium uses 10 fishes. The study container layout was arranged at random to reduce the biased in each treatment. The parameters observed included the number of white blood cells, red blood cells, hematocrit and water quality.

Preparation of Containers

Aquarium as a container for the maintenance of test fish as much as 12 pieces, with size 40 x 25 x 25 cm^3, where 9 pieces for the treatment and 3 pieces as a aquarium stock equipped with blowers, hoses and aerated stone prepared first. Before use it, the aquarium was washed first and then soaked with chlorine as much as 30 ppm for 24 hours then rinsed with clean water and dried. A sterile aquarium is filled with water then as much as 15 liters and aerated.

Test Fish Adaptation

Test fish used are common carp, nilem and nilem padjadjaran strain, size 8-12 cm. Common carp and nilem obtained from Fish Seed Center in Bayongbong, Garut. Nilem padjadjaran strain obtained from Experimental Pond of Fishery and Marine Sciences Faculty, Universitas Padjadjaran. The test fish was acclimatized for 14 days to uniform the condition. During the acclimation period, fish are fed 2 times daily in adlibitum.

Provision of Isolate *A. Hydrophila*

Isolate of *A. hydrophila* bacteria used in this research is the cultivation result of biotechnology laboratory and molecular of Universitas Padjadjaran which originally came from Bandung Institute of Technology. Prior to use for the challenge test, *A. hydrophila* increased its malignancy by injection to fish in the intraperitoneal portion of 0,2 ml/head. Fish that have been infected by *A. hydrophila*, observed until clinical symptoms appear. Fish that have shown clinical symptoms are then isolated from the skin of bacteria, eyes, liver and kidney. The growing colony was observed for its morphology and compared to the colony present in the initial isolate stock.

White Blood Cells (WBCs) Calculation

Fish sampling of single fish in each aquarium before the challenge test. Taking blood fish by putting fish on wet cloth to facilitate the blood taking, then, fish slashed at the base of the tail until the blood out. Blood is taken on the slashed section using a thomma pipette up to a scale of 0,5. Then continued by sucking the Turk solution up to scale 11 and homogenized by shaking the thomma pipette. The resulting solution is introduced into the haemocytometer and covered with a cover glass. Observations and calculations were performed using a microscope with 100x magnification. White blood cells is calculated by the formula (Nabib and Pasaribu 1989):

$$\Sigma \text{ WBCs per ml of blood} = \text{Average number of WBCs} \times \text{multiplier factor}$$

$$\text{Multiplier factor} = \text{dilution vol.} \times \text{Thickness of haemocytometer} \times \text{Number of squares}$$

Information :

Dilution volume	: 20
Thickness of haemocytometer	: 10
Number of squares	: 16

Red Blood Cells (RBCs) Calculation

Fish sampling of 1 fish in each aquarium before the challenge test. Taking fish blood by putting fish on wet cloth to facilitate the blood taking, then fish slashed at the base of the tail until the blood comes out. Blood is taken on the slashed part using a pipette thomma pipette thomma up to a scale of 0,5. Then proceed by sucking the Hayem solution to scale 101 on the thomma pipette and homogenized by shaking the thomma pipette. The resulting solution is introduced into the haemocytometer and covered with a cover glass. Observations and calculations were performed using a microscope with 100x magnification. The calculation of white blood cells is calculated by the formula (Nabib and Pasaribu 1989):

$$\Sigma \text{ RBCs per ml of blood} = \text{Average number of WBCs} \times \text{multiplier factor}$$

$$\text{Multiplier factor} = \text{dilution vol.} \times \text{Thickness of haemocytometer} \times \text{Number of squares}$$

Information :

Dilution volume	: 200
Thickness of haemocytometer	: 10
Number of squares	: 25

Measurement of Hematocrit

The hematocrit is measured by inserting blood samples into a microhematocrit capillary tube up to 3/4 tubes. Capillary microhematocrit in centrifuge for 4 minutes with a speed of 12.000 rpm. The erythrocyte sediment was measured in length using a hematocrit reading chart to determine its hematocrit level.

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RESULT AND DISCUSSION

Number of White Blood Cells

Observation of white blood cells count was done to determine the change of white blood cells count in common carp, nilem and nilem padjadjaran strain after challenge tested with *A. hydrophila*. The decrease in fish resilience can be seen through changes in the blood picture of fish, especially the total number of white blood cells (Mumpuni and Mulyana 2016). Therefore, this is the basis for taking precautions against bacterial or parasitic infections when there is an indication of decreased endurance of fish body. The average number of white blood cells in common carp, nilem and nilem padjadjaran strain before the challenge test and post-challenge test is presented in Figure 1 below.

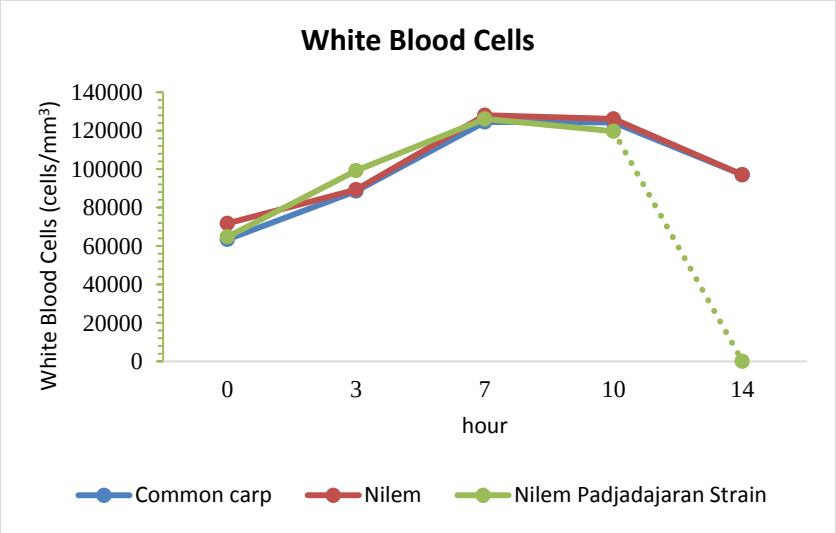

Figure 1. Average number of white blood cells

According to Fig. 1, the zero hour is the pre-challenge treatment, the average number of white blood cells of common carp, nilem and nilem padjadjaran strain respectively are 63.467, 71.733 and 64.800 cells/mm^3. According to Lagler (1977) the total white blood cells in freshwater fish ranged from 20.000 - 150.000 cells/mm^3. Based on research of Dianti et al. (2013), white blood cells count is 27.300 cells/mm^3, then based on Mulyana and Mumpuni (2016) research, the number of white blood cell of nilem fish is 70.500 cells/mm^3. These results indicate that white blood cells of nilem fish are higher than common carp.

After the challenge test at the third hour until the seventh, the average white blood cell of common carp, nilem and nilem padjadjaran strain experience the same with a relatively similar pattern. The increasing number of white blood cells indicates the presence of infectious diseases caused by *A. hydrophila* bacteria that are infected into the body of the test fish. *A. hydrophila* infected test fish will produce more white blood cells to phagocytes the bacteria as a form of body defense (Moyle and Cech 2004).

A decrease in the number of white blood cells occurs in the tenth to the fourteenth hour after the challenge test. However, after the passing of the tenth hour, the nilem padjadjaran strain fish has experienced total mortality indicated by the dashed line in Fig. 1, while some common carp and nilem still live with a relatively similar pattern of white blood cell loss. The decline in the number of white blood cells is suspected test fish are not able to resist from of A. hydrophila bacteria attack that was initially infected into the body as much as 10^8cfu/ml. Hastuti et al. (2012) revealed that fish infected with the disease will decreased white blood cells caused by disruption of kidney and lymph function in producing white blood cells. This makes the ability of white blood cells to decrease because white blood cells serve as a non-specific defense that eliminates pathogens.

Table 1. Test fish's white blood cells

Hour	Treatment	Average
0	Common carp	63.467 ± 2.013[a]
	Nilem	71.733 ± 4.822[b]
	Nilem padjadjaran strain	64.800 ± 2.884[a]
3	Common carp	88.533 ± 1.222[a]
	Nilem	89.333 ± 5.327[a]
	Nilem padjadjaran strain	99.200 ± 3.666[b]
7	Common carp	124.533 ± 5.081[a]
	Nilem	128.000 ± 5.600[b]
	Nilem padjadjaran strain	126.133 ± 9.272[a]
10	Common carp	124.267 ± 5.787[a]
	Nilem	126.133 ± 2.444[b]
	Nilem padjadjaran strain	119.733 ± 2.810[a]
14	Common carp	97.067 ± 3.233[a]
	Nilem	97.067 ± 1.665[a]
	Nilem padjadjaran strain	0

Based on the data presented in Table 1, the number of white blood cells of dominant nilem fish was significantly different from white blood cell of common carp and nilem padjadjaran strain. This is made a hypothesis that the hybridization results between the common carp and the nilem fish this makes the breed endurance follow the common carp. The inheritance nature of blood cells is thought due to the genes dominance in common carp is stronger than the nilem fish so that the hybrid results lead to common carp. This result is consistent with the research of Simkova et al. (2014) that the morphology in the hybrid fish *Cyprinus carpio* x *Carrasius gibel* have an intermediate character, whereas for the biochemical character and immune system of the hybrid fish are still similar to *Cyprinus carpio*, in which case the common carp becomes its male parent. In addition there is a hypothesis that hybrid fish show weakened body resistance due to genetic mismatch of both parent species (breakdown of parent genes interfering in hybrids). Research on the blood picture of nilem padjadjaran strain has similarities to studies conducted by Simkova et al. (2014), that is the test fish used are from one family (Cyprinidae) and the males used from the same species, *Cyprinus carpio*.

Its generally known that hybridization results are superior compare to those of the two in various performance. However, it should be understood also that the actual results of hybridization does not always lead to improving the quality of offspring. The results of this crosses are highly variable and conditioned by many factors, including the biology of parental lines and the heterozygosity of the crosses (Andriasheva 2013).

Number of Red Blood Cells

Red blood cells counts observation was done to determine the red blood cells count in common carp, nilem and nilem padjadjaran strain after tested with *A. hydrophila* bacteria. Zainun (2007) states that one of the indicators to determine the condition of fish health is through the blood profile of the fish. Fish infected with bacteria or diseases will experience changes in the amount of erythrocytes, leukocytes and hemoglobin concentrations. Therefore, the observation of red blood cells (erythrocytes) held to determine whether A. hydrophila infecting fish will affect the blood profile of goldfish, nilem and nilem padjadjaran. Here is the average red blood cell test fish presented in Figure 2.

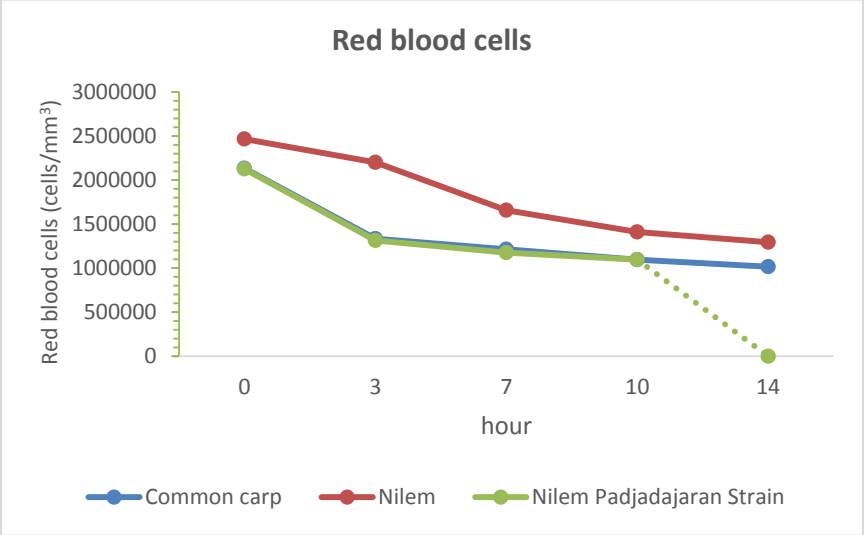

Figure 2. Average number of red blood cells

Fig. 2 shows the average red blood cells of common carp, nilem and nilem padjadjaran strain before the challenge test (zero hour) is 2.133.333, 2.466.667 and 2.126.667 cells/mm^3. According to Irianto (2005) the number of erythrocytes in teleostei fish ranged from 1.050.000 – 3.000.000 cells/mm^3. Based on research by Dianti et al. (2013), the number of red blood cells of common carp is 2.980.000 cells/mm^3, then based on Mulyana and Mumpuni (2016) research, the number of red blood cells in nilem fish is 2.030.000 cell/mm^3.

After the challenge test at third hours, the mean of red blood red fish, nilem and nilem padjadjaran strain decreased sequentially to 1.333.333, 2.200.000 and 1.313.333 cells/mm^3. The decrease in red blood cell count lasted until the 14th hour after the challenge test with the result of a relatively similar pattern of decrease. The decrease of red blood cells indicates that the test fish have been infected by *A. hydrophila* bacteria. According to Haditomo et al. (2014) the decline of red blood cells is considered to be one of the attack impact by *A. hydrophila* bacteria. Entering the 14th hour after the test challenge the decrease turn into 1.016.667 cells/mm^3 for common carp and 1.293.333 cells/mm^3 for the nilem, while the nilem padjadjaran strain has experienced total death indicated by the dashed line in Fig. 2, so no calculation of blood cells. Differences in the number of red blood cells from the three test fish before the challenge test and post-challenge to *A. hydrophila* is due to the lysis of red blood cells. Haditomo et al. (2014) also mentioned that the process of rupture of red blood cells due to toxin produce bacteria in the form of haemolisin enzymes play a role to lyse red blood cells.

Table 2. Test fish red blood cells

Hour	Treatment	Rata-rata
0	Common carp	2.133.333 ± 110.151^a
	Nilem	2.466.667 ± 63.509^b
	Nilem padjadjaran strain	2.126.667 ± 49.329^a
3	Common carp	1.333.333 ± 60.277^a
	Nilem	2.200.000 ± 36.056^b
	Nilem padjadjaran strain	1.313.333 ± 25.166^a
7	Common carp	1.213.333 ± 50.332^a
	Nilem	1.656.667 ± 118.462^b
	Nilem padjadjaran strain	1.176.667 ± 50.332^a
10	Common carp	1.096.667 ± 63.059^a
	Nilem	1.410.000 ± 30.000^b
	Nilem padjadjaran strain	1.096.667 ± 49.329^a
14	Common carp	1.016.667 ± 35.119^a
	Nilem	1.293.333 ± 179.536^b
	Nilem padjadjaran strain	0

Based on the data presented in Table 2, the number of red blood cells of nilem fish is always significantly different from red blood cells of common carp and nilem padjadjaran strain. These results did not vary greatly with the results of further tests on white blood cells from all three test fish.

Hematokrit

Hematocrit observation was done to determine the effect of *A. hydrophila* attack seen from the percentage change and this result can describe health condition of test fish. The average hematocrit of the test fish presented on Figure 3.

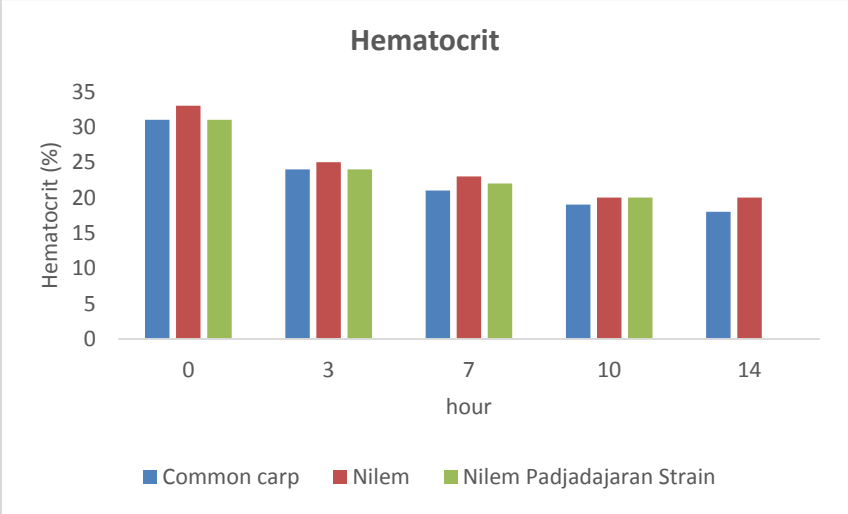

Figure 3. Average number of Hematocrit

According to Fig. 3, before the challenge test (zero hour) the hematocrit level of common carp, nilem and nilem padjadjaran strain are 31%, 33% and 31%, respectively. Based on Mulyana and Mumpuni (2016) hematocrit common carp ranges from 28 - 40%. Entering the third hour to 14 hours hematocrit levels always decrease in every hour of observation. Decrease in hematocrit levels is suspected caused by the fish experience stress due to *A. hydrophila* attack. According to Dianti et al. (2013) decreased levels of hematocrit is a clue due to low protein, vitamin or fish deficiency are infected. The same is also stated by Mulyana and Mumpuni (2016) that fish affected by infection, appetite will decrease and the value of hematocrit will decrease.

Water Quality

Water quality observations were used as supporting parameters during the study. The condition of water quality as a maintenance medium of common carp, nilem and nilem padjadjaran strain very influential on the fish immune system during the study. Water quality observation results can be seen in Table 3.

Table 3. Water quality

Treatment	Parameters of Water Quality		
	Temperature (^{o}C)	pH	DO (mg/l)
Common carp	24,3-24,5	7,29-7,85	5,4-5,5
Nilem	24,2-24,5	7,49-7,93	5,4-5,5
Nilem padjadjaran strain	24,2-24,4	7,47-8,03	5,5-5,6
Optimal	20-28 (Serdiati 1988)	6,5-8,5 (SNI)	≥5 (SNI)

Based on Table 3, it is seen that the average temperature of each treatment is in the range of 24,2 - 24,5°C, average pH 7,29 – 8,03 and the average number of dissolved oxygen content is 5,4 - 5,6 mg/l. Based on the results of water quality measurements during the study, the temperatures, pH and DO are within the optimal range and meet the optimum standards in accordance with Serdiati (1988) and Indonesian National Standard (SNI) 01- 6133 - 1999 for the maintenance of common carp.

CONCLUSION

Based on the results of research that has been done then it can be concluded that white blood cells and red blood cells are not significantly different with common carp. The number of white blood cells of common carp, nilem and nilem padjadjaran strain before the challenge test were 63.467, 71.733 and 64.800 cells/mm^3 respectively, while the number of white blood cells after tenth hour test is 124.267, 126.133 and 119.733 cells/mm^3. The number of red blood cells of common carp, nilem and nilem padjadjaran strain before the challenge test is 2.133.333, 2.466.667 and 2.126.667 cells/mm^3, while tenth hour post-test becomes 1.096.667, 1.410.000 and 1.096.667 cells/mm^3. Hematocrit of common carp, nilem and nilem padjadjaran strain before the challenge test that is 31%, 33% and 31%, while hematocrit post-challenge test turned into 19%, 20% and 20%.

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REFERENCES

[1] Abidin. 2013. *Efektivitas Penggunaan Sperma Ikan Mas (Cyprinus carpio) Terhadap Keberhasilan Ginogenesis dan Hibridisasi Ikan Nilem* (*Osteochilus hasselti*). Skripsi. FPIK Unpad. Jatiangor.

[2] Andriasheva. 2013. *Commercial Hybridization and Heterosis in Fish Culture*. Scientific Research Institute on Lake and River Fisheries Leningrad, U.S.S.R. http://www.fao.org/docrep/005/b3310e/B3310E18.htm (diakses pada tanggal 24 April 2018 pukul 09.12 WIB).

[3] Arianto dan Utami. 2006. Evaluasi Laju Pertumbuhan, Keragaman Genetik dan Estimasi Heterosis pada Persilangan Antar Spesies Ikan Patin (*Pangasius* sp.). *Jurnal Perikanan*. 8 (1) : 81-86.

[4] Dianti, Prayitno dan Ariyati. 2013. Ketahanan Nonspesifik Ikan Mas (*Cyprinus carpio*) yang Direndam Ekstrak Daun Jeruju (*Acanthus ilicifolius*) Terhadap Infeksi Bakteri *Aeromonas hydrophila*. *Journal of Aquaculture Management and Technology*. 2 (4) : 63 – 71.

[5] Haditomo, Widanarni dan Lusiastuti. 2014. *Perkembangan Aeromonas hydrophila pada Berbagai Media Kultur*. Seminar Nasional Ke-III : Hasil-Hasil Penelitian Perikanan dan Kelautan. Semarang : Undip.

[6] Hastuti, Minaka dan Sarjito. 2012. Identifikasi Agensia Penyebab dan Profil Darah Ikan Gurami (*Osphronemus gouramy*) yang Terserang Penyakit Bakteri. *Journal of Aquaculture Management and Technology*. 1 (1) : 249-263.

[7] Irianto. 2005. *Patologi Ikan Teleostei*. Yogyakarta: Gadjah Mada University Press.

[8] Kordi. 2004. *Penanggulangan Hama dan Penyakit Ikan*. PT Rineka Cipta dan PT Bina Adiaksara. Jakarta.

[9] Lagler, K. F., J. E. Bardach., R. R. Miller., D. R. M. Passino. 1977. *Ichtiology*. John Wiley & Sons, Inc. United State of America.

[10] Manurung dan Susantie. 2017. Identifikasi Bakteri Patogen pada Ikan Nila (*Oreochromis Niloticus*) di Lokasi Budidaya Ikan Air Tawar Kabupaten Kepulauan Sangihe. *Jurnal Budidaya Periaran*. 5 (3) : 11 – 17.

[11] Moyle dan Cech. 2004. *Fish An Introduction To Ichthyology*. Fifth Edition. New Jersey: Prentice Hall.

[12] Mulyadi, G.K., Buwono dan Subhan. 2017. Analisis Kekerabatan Genetik Hibrid Ikan Nilem (*Osteochilus hasselti*) dan Ikan Mas (*Cyprinus carpio*) Menggunakan PCR-RAPD. *Jurnal Perikanan dan Kelautan*. 8 (1) : 42-47.

[13] Mulyana dan Mumpuni. 2016. Gambaran Darah Ikan Nilem yang Diuji Tantang dengan *Aeromonas hydrophila*. *Prosiding Seminar Nasional Masif II.* 144-151.

[14] Mulyasari. 2010. *Karakteristik Fenotipe Morfomeristik dan Keragaman Genotipe RAPD (Randomly Amplified Polymorphism DNA) Ikan Nilem (Osteochilus hasselti) di Jawa Barat*. Tesis. Institut Pertanian Bogor.

[15] Nabib, R. dan Pasaribu F.H. 1989. *Patologi dan Penyakit Ikan*. Departemen Pendidikan dan Kebudayaan. Direktorat Jendral Pendidikan Tinggi. Pusat Antar Universitas Bioteknologi. Institut Pertanian Bogor.

[16] Rosidah. 2016. *Parasit dan Penyakit Ikan*. Bandung : Unpad Press.

[17] Serdiati. 1988. *Pengaruh Padat Penebaran Terhadap Pertumbuhan Ikan Mas (Cyprinus carpio) yang Dipelihara dalam Karamba pada Kolam dengan Input Air Limbah Rumah Tangga*. Skripsi. Fakultas Peternakan. Universitas Hasanudin. Ujung Pandang.

[18] Simkova, A., Vojtek, Halacka, Hyrsl and Vetesnik. 2014. *The effect of hybridization on fish physiology, immunity and blood biochemistry: A case study in hybridizing Cyprinus carpio and Carassius gibelio (Cyprinidae).* doi: 10.1016/j.aquaculture.2014.10.021 (diakses pada tanggal 19 Mei 2018 pukul 09.34 WIB)

[19] SNI 01- 6133 – 1999. *Produksi Benih Ikan Mas (Cyprinus carpio Linneaus) strain Majalaya Kelas Benih Sebar*. BSN.

[20] Triyaningsih, Sarjito dan 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.

[21] Zainun. 2007. *Pengamatan Parameter Hematologis pada Ikan Mas yang diberi Immunostimulan.* Teknisi Litkayasa pada Balai Besar Pengembangan Budidaya Air tawar. Sukabumi.