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Hematological Performance and Survival of Common Carp (*Cyprinus carpio* Linnaeus, 1758) Strain Majalaya Fingerlings Against Temperature Stress

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ABSTRACT

The research was conducted at the Aquaculture Laboratory of the Faculty of Fisheries and Marine Science, Universitas Padjadjaran, Jatinangor. The purpose of this research is to determine the optimum temperature for the maintenance of Common carp strain Majalaya fingerlings based on resistance to temperature stress that is known through their blood profile and survival rate. Common carp strain Majalaya fingerlings originating from Ciparay, West Java. Total body length is 8-12 cm with a minimum weight of 10 g. The method used is experimental with a completely randomized design (CRD) consisting of three treatments and five replications. The fish are kept at 24 ºC (control without heater), 29 ºC and 34 ºC. Each experimental unit contains 10 fishes. The results obtained by the survival rate (SR) of Common carp strain Majalaya which were maintained at a treatment temperature of 24 ºC by 100%, the 29 ºC by 70%, the 34 ºC by only 42% remained on the 21st day. The number of erythrocytes of common carp strain Majalaya fingerlings before the treatment temperature was 2.77×10^6 cells/mm^3. The average number of erythrocytes of the 24ºC treatment from 7th, 14th and 21st day were 2.82, 2.82 and 2.81×10^6 cells/mm^3; the 29 ºC were 2.93, 2.68 and 2.42×10^6 cells/mm^3; the 34 ºC were 3.08, 2.52 and 1.99×10^6 cells/mm^3. While the number of leukocytes of common carp strain Majalaya fingerlings before the treatment temperature was 13.61×10^4 cells/mm^3. The average number of leukocytes of the 24 ºC treatment from 7^{th}, 14^{th} and 21^{st} day were 13.73, 13.71 and 13.88×10^4 cells/mm^3; the 29 ºC were 18.40, 16.46 and 15.76×10^4 cells/mm^3; the 34 ºC were 20.78, 18.46 and 16.81×10^4 cells/mm^3.

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***Keywords*:** Common carp Strain Majalaya, maintenance temperature, survival rate, number of erythrocytes, number of leukocytes, *Aeromonas hydrophila, Cyprinus carpio, C. c. haematopterus*

1. INTRODUCTION

The common carp (*Cyprinus carpio*) is one of the oldest domesticated freshwater species with two highly divergent subspecies *C. carpio* from Europe/Central Asia and *C. c haematopterus* from East Asia, as confirmed by current molecular genetic studies. Numerous productive populations, breeds, and strains distinguishable by means of nuclear and mtDNA markers have been developed from both ancestral subspecies, as well as from their mutual hybrids and backcrosses followed by mass selection.

Common carp is a very popular fishery commodity and is a type of consumption fish that is much in demand by the Indonesian people because it is easily available, the meat is tender and the taste is tasty. Common carp that have been cultivated in Indonesia are known to have several strains, namely Punten, Sinyonya, Domas, Merah/Cangkringan, Kumpai, Majalaya and so on. Common carp strain Majalaya has several advantages including its relatively fast growth, resistance to *Aeromonas hydrophila* bacterial infection and fecundity or the number of eggs produced is quite high, range between 84,000-110,000 eggs per kilogram of parent.

One of the obstacles in the business of Common carp farming is the low survival rate and relatively slow growth of fish due to adverse factors that affect their health conditions caused by environmental changes such as temperature, salinity, changes in water pressure, pollution, pH, changes in water flow, sediment loads, DO concentrations and feed availability. Temperature is one of the main factors causing fish to be stressed. Stress in fish is a condition in which fish are unable to regulate normal physiological conditions in their bodies. This stress will have an impact on decreased appetite and stunted fish growth.

Temperature is an important factor for fish growth and survival. Temperature affects the survival of fish, from eggs, seeds to adult size. The tolerance range and optimum temperature of fish breeding sites are different for each fish species/species, to different growth stages. The optimum temperature for common carp growth is in the range of 25 - 30 °C. While Common carp strain Majalaya have water temperature tolerance in the range 18 - 32 °C.

Tropical waters have a water temperature of 25 - 30 °C while lakes in the tropics have a high temperature range of between 20 - 30 °C. According to the water quality data of the Ciparay Fish Fingerlings Center, West Java, the measurement of the water temperature in Ciparay ranged from 24.2 °C to 29.3 °C.

The large temperature range of these waters allows for ineffective maintenance of Common carp strain Majalaya if done in a place that has high water temperature fluctuations. Water temperature affects the hematological parameters and resistance to disease. Therefore, need to know the optimum water temperature for maintaining Common carp strain Majalaya fingerlings. Indicators of optimum water temperature for maintenance can be seen from the survival and blood profile of fish. Blood characteristics can be used to evaluate the physiological response in fish. Water temperature that is not optimal will result in a low survival rate and abnormal blood profile values that indicate the fish are experiencing stress. The stress response in fish can be seen from its erythrocytes and leucocytes count. Stress conditions cause changes in the erythrocyte count, hematocrit and hemoglobin counts, while the leucocyte count tends to increase. Increase in water temperature causes an increase in immune response in fish. Stress due to high temperature can increase fish erythrocyte count.

2. MATERIAL AND METHOD

Experimental method is used with Completely Randomized Design (CRD) conducted with three treatments and five replications. The aquarium is divided into five parts with the dimensions of each part of 20 cm × 40 cm × 40 cm with a 24 liters volume of water. The aquarium is set and then filled with water as much as 75% of the volume. Treatment A: fish kept at 24 ± 1 ºC (control without heater); treatment B: fish kept at a temperature of 29 ± 1 ºC; treatment C: fish kept at 34 ± 1 ºC. Each aquarium contains 10 Common carp Strain Majalaya fingerlings, 8-12 cm body length, with 10 g weight average. The temperature was raised slowly (2 ºC/day) from 24 ºC (control/without heater) to 29 ºC for treatment B and 34 ºC for treatment C for about 5 days then acclimation for 2 days until they adapted. The feed used for this study was fish pellets containing 30-32% protein by feeding 5% of fish biomass and feeding frequency three times a day. Turk solution is a white blood thinning solution made from glacial acetic acid 2.5% 15 ml, gentian violet 1 ml and distilled water 475 ml. Hayem's solution is used to thin red blood cells.

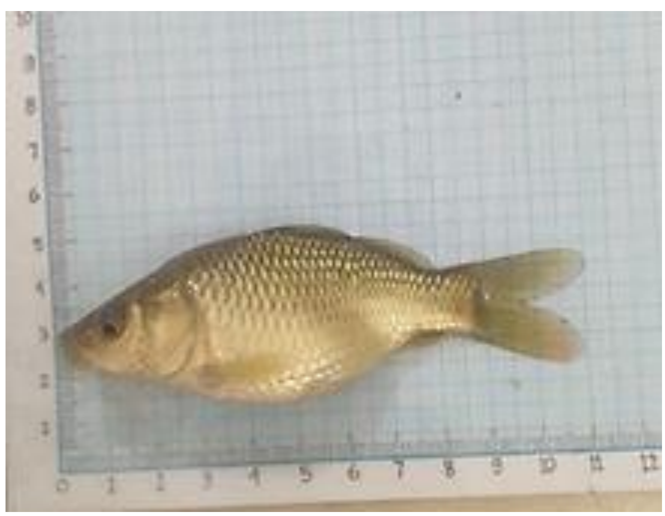

Figure 1. Common Carp Majalaya Fingerlings

2. 1. Observation Parameters

Each seven days, on the 7th day, 14th and 21st, pH and DO, survival rate (SR) calculations and blood samples were taken in the laboratory to count the number of erythrocytes and leukocytes from each fish in the treatment replication.

a) Survival Rate (SR)

Survival Rate of Common carp strain Majalaya fingerlings are calculated at the end of the study calculated using the formula:

$$SR = \frac{Nt}{No} x\ 100\%$$

Information : SR = Survival Rate (%)
Nt = Number of live test fish at the end of the observation
No = Number of test fish at the beginning of the observation

b) Number of Erythrocytes and Leukocytes

- **Erythrocyte**

Calculations are made on 5 large haemocytometer boxes and the amount is calculated by the formula:

$$\Sigma \text{ Erythrocyte/ml} = \text{Number of erythrocyte } x \text{ multiplier factor}$$

*multiplier factor = 200 *x* 10 *x* $(5)^2$
Information:
Dilution volume = 200
Haemocytometer thickness = 10
Number of boxes = 25

- **Leukocyte**

Calculations are made on 4 haemocytometer boxes and the amount is calculated by the formula:

$$\Sigma \text{ leukocyte/ml} = \text{Number of erythrocyte } x \text{ multiplier factor}$$

*multiplier factor = 200 *x* 10 *x* $(4)^2$
Information:
Dilution volume = 20
Haemocytometer thickness = 10
Number of boxes = 16

c) Daily Growth Rate

Daily growth rate is calculated by the formula:

$$\Delta G = (Wt - Wo) / t$$

Information:
ΔG = Growth (g),
Wt = Fish weight at the end of study (g),
Wo = Fish weight at the beginning of study (g).
t = research time (day)

d) Water Quality

Measurements were taken four times during the study. The first sampling just before the study, the second sampling on the 7th day, the third sampling on the 14th day, and the fourth sampling on the 21st day. The measurement of pH used a pH meter, while the measurement of DO used a DO meter.

2. 2. Data Analysis

Survival rate data, the number of erythrocytes and leukocytes and daily growth rate were analyzed using the F (ANOVA) test, if there were significant differences between treatments then continued by Duncan test with a 95% of trust level. While water quality was analyzed descriptively.

3. RESULT AND DISCUSSION

3. 1. Survival Rate

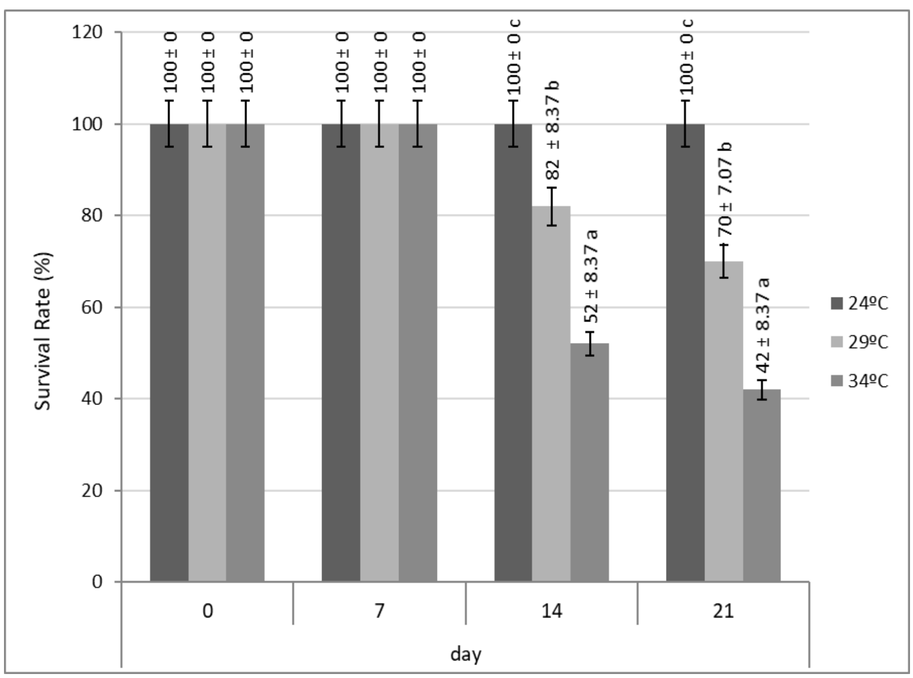

Figure 2. Survival Rate

Survival rate is the ratio between the number of individuals that alive at the end of rearing with the number of individuals that alive at the start of rearing. Survival rate can be used as a measure of the success of fish maintenance until the end of the study.

Figure 2 shows that the survival rate of Common carp Majalaya fingerlings with a treatment temperature of 24 ºC has 100% survival rate from the early until the end of the study. The 29 ºC temperature treatment has 100% survival rate value on the 7th day, but it decreased to 82% on the 14th day, on the 21st day the value continued to decrease to 70% due to fish mortality. The lowest Survival rate value was from the highest temperature treatment, 34 ºC, on the 7th day all fishes were still survived with 100% survival rate, but on the 14th day it dropped drastically to 52% with the number of fish were deaths. It continued to decrease to 42% due to fish mortality. This shows that the higher treatment temperature is inversely proportional to the survival rate of the Common carp Majalaya fingerlings.

The treatments of 29 ºC and 34 ºC has the lower and lowest survival rate percentage due to fish mortality. This was happened because the fish in theese treatments were stress while adapting in higher temperature for long time. The high range temperature treatments that given in theese treatments by 5 ºC causing large impact towards the fish that could not survive would die and the otherwise.

In this case, based on the survival rate data in Fig. 2, it shows that the survival of Common carp Majalaya fingerlings maintained in aquariums with a treatment temperature of 24 ºC is the best treatment with a survival rate reaching 100% followed by the 29 ºC by 70% and the 34 ºC by 42% at the end of the study. This shows that the higher the temperature, the lower the survival rate of Common carp Majalaya fingerlings.

3. 2. Number of Erythrocyte

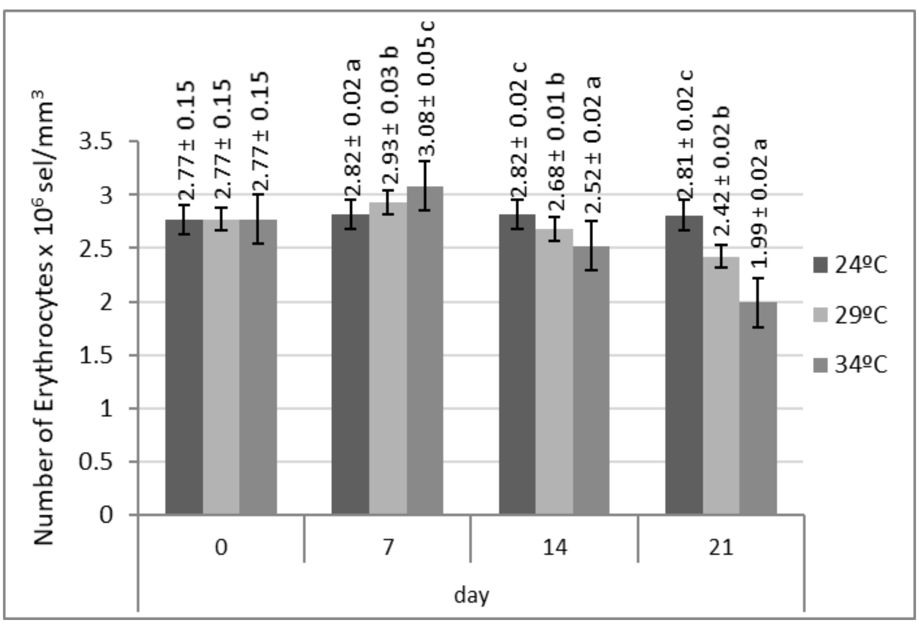

Figure 3. Average Number of Erythrocytes

Figure 3 shows the average number of erythrocyte counts of Common carp Majalaya fingerlings on first sampling (before given temperature treatments = day 0) is 2.77×10^6 cells/mm^3. The number of erythrocytes in teleost fish ranged from 1.05 - 3.0×10^6 cells/mm^3.

The second sampling was carried out on the 7th day of the study, the results of the three temperature treatments 24 °C, 29 °C and 34 °C were significantly different ($P<0.05$) among treatments with an average erythrocyte value of 2.82, 2.93 and 3.08 × 106 cells/mm^3. This shows an increase in the number of erythrocytes which is directly proportional to the higher temperature treatment. Increasing temperature, the number of erythrocytes increases.

When the temperature increases, the activity of oxygen absorption and erythrocytes increases. The fish's body compensates for this change in oxygen deficiency by increasing the number of erythrocytes. According to Ravichandra (2012) that at high temperatures the number of erythrocytes in fish increases because to reduce stress, the fish will adjust their physiological conditions by increasing the number of erythrocytes in the blood.

The third sampling was carried out on the 14th day and the results were significantly different among the treatments with the average number of erythrocytes treated at 24 °C, 29 °C and 34 °C are 2.82, 2.68 and 2.52 × 10^6 cells/mm^3. The results of this sampling showed that the higher the temperature, the lower the number of erythrocytes. It is different from the previous sampling which is the 7th day which shows the opposite.

The fourth sampling was carried out at the end of the study or the 21st day where it was seen from the average number of erythrocytes of Common carp Majalaya fingerlings that were maintained at the three treatment temperatures, 24 ºC, 29 ºC and 34 ºC, which were significantly different among treatments ($P<0.05$) with the average erythrocyte values of 2.81, 2.42 and 1.99 × 10^6 cells/mm^3. These results show the similarity of the phenomenon to the results of the 14th day of sampling, the higher the temperature, the lower the number of erythrocytes.

Based on the results of the 7th, 14th and 21st day sampling, it can be seen that the 7th day of sampling, which shows the higher the temperature, the higher the number of erythrocytes because on the 7th day it can still be said to be the initial stage of fish adapting to the treatment temperature so that it is natural if the number of erythrocytes increases (Ravichandra 2012).

Sampling on days 14 and 21 shows the opposite phenomenon, the higher the temperature, the lower the number of erythrocytes. The research was conducted over a long period of time (over 15 days), showing that the increasing water temperature can reduce the number of fish erythrocytes at the end of the study. The decrease in the number of erythrocytes is the response of the fish's body which began to adapt to the treatment temperature where initially the number of erythrocytes increased as on day 7 because the fish were still experiencing stress due to the high treatment temperature. The number of erythrocytes gradually decreased on day 14 and 21, indicating that the stress in fish began to decrease and the fish's body had begun to adapt to the temperature of the treatment.

Based on Figure 3, it shows that the number of erythrocytes at 24 °C treatment on the 7th, 14th, and 21st-day sampling was 2.82, 2.82, and 2.81 × 10^6 cells/mm^3. This shows that the number of erythrocytes of Common carp Majalaya fingerlings which were maintained in 24 ºC is still within the normal range for the number of common carp erythrocytes (2.98 × 10^6 cells/mm^3) and there is no significant change so that the fish were in good condition.

Whereas in the treatment temperatures of 29 ºC and 34 ºC there are increasing in the number of erythrocytes on the 7th day and then a decrease in the number on the 14th day until the 21st day. The increase in the number of erythrocytes on 7th day due to high temperatures is caused by the level of dissolved oxygen in the water which is getting lower so that the fish's body produces more erythrocytes to get enough oxygen. Whereas the decrease in the number of erythrocytes on 14th and 21st day shows that the fish have begun to adapt to the treatment temperature, but the reduction in the number of erythrocytes to 1.99 × 10^6 cells/mm^3 at the treatment temperature of 34 ºC is very significant and far adrift from normal range of common carp fish erythrocytes so that the fish may be anemic or damage to the formation of blood cells in fish found in the liver and kidneys of fish which are a place of hematopoiesis in fish. High temperature treatment can also cause damage in the form of necrosis, fibrosis and hemorrhage in fish kidneys (Ibrahim 2013).

3. 3. Number of Leukocyte

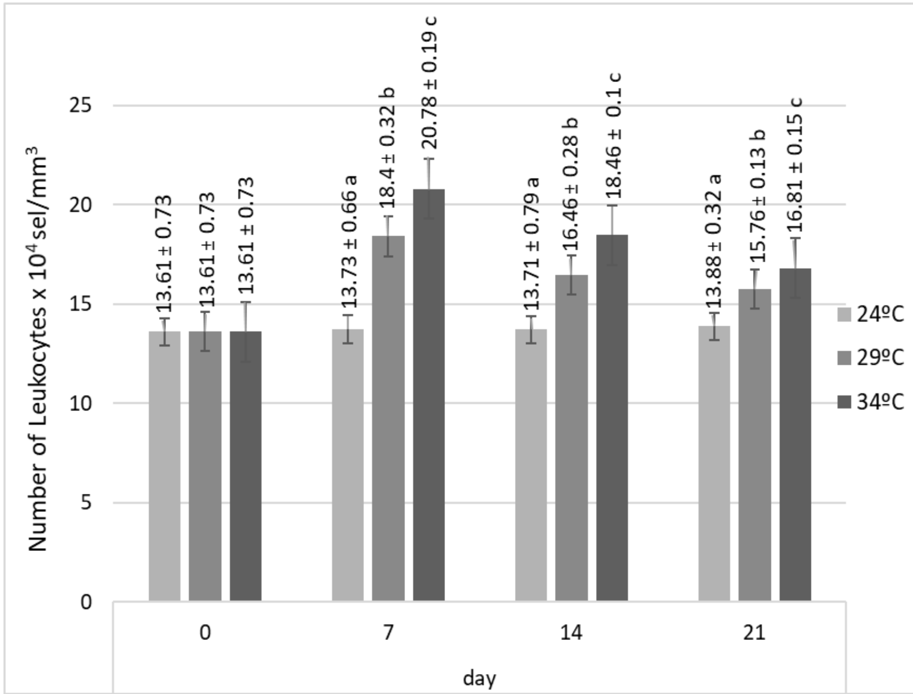

Figure 4. Average Number of Leukocytes

Figure 4 shows that the more the temperature increases the more the number of leukocytes. On the first sampling (before given the temperature treatment = day 0) the average number of leukocytes in Common carp Majalaya fingerlings was 13.61 × 10^4 cells/mm^3. The number of leukocytes in freshwater fish ranged from (2 - 15) × 10^4 cells/mm^3 and the average number of healthy common carp leukocytes is 12.55 × 10^4 cells/mm^3.

The second sampling carried out on the 7th day showed that the three treatments were significantly different ($P<0.05$). The average number of leukocytes from the temperature of 24 ºC, 29 ºC, and 34 ºC, respectively 13.73, 18.40, and 20.78 × 10^4 cells/mm^3. This value indicates that the higher the temperature, the more the leukocyte count increases.

The third sampling, on day 14 also showed significantly different numbers among treatments ($P<0.05$) with the average number of leukocytes at treatment temperatures of 24 ºC, 29 ºC and 34 ºC, respectively 13.71, 16.46 and 18.46 × 10^4 cells/mm^3. The leukocyte count here also shows the higher the temperature the higher the leukocyte count.

The fourth sampling on the 21st day still showed significant differences among treatments ($P<0.05$). The average number of leukocytes from the treatment temperature 24 ºC, 29 ºC and 34 ºC were 13.88, 15.76 and 16.81 × 10^4 cells / mm^3. Similar to the 7th and 14th-day sampling, this time sampling also showed that the higher the temperature the higher the leukocyte count in the Common carp Majalaya fingerlings.

The increase in the number of leukocytes indicates the fish's immune response to stress due to high water temperatures so that the body's defenses and antibody production also increases. The increase in the number of leukocytes also shows that the fish may be infected, stress, or experiencing leukemia. In fish, an increase in water temperature can increase the number of its leukocytes. Leukocyte cells can increase due to an increase in water temperature, if the water temperature increases, the activity of fish's body defense cells also increases.

Figure 4 shows the 24 ºC treatment temperature, the average number of leukocytes on Common carp Majalaya fingerlings from the 7th day to the 21st day at the end of the study, known the number of leukocytes respectively 13.73, 13.71 and 13.88 × 10^4 cells/mm^3 shows a consistent number and no significant changes. This means describing the condition of a good fish and normal. The number of leukocytes is still within the normal range of freshwater fish (2-15 × 10^4 cells/mm^3). Whereas the number of leukocytes at 29 ºC and 34 ºC treatment temperature increased on the 7th day and then continued to decrease until the 21st day. The 29 ºC treatment temperature has the average number of leukocytes on Common carp Majalaya fingerlings from the 7th day to the 21st day at the end of the study respectively by 18.40, 16.46 and 15.76 × 10^4 cells/mm^3 while the 34 ºC treatment temperature by 18.40, 16.46 and 15.76 × 10^4 cells/mm^3.

The increase in the number of erythrocytes is the fish's response to stress due to high temperatures so that the fish's body increases the number of leukocytes in its blood. The decrease in the number of leukocytes is suspected because the fish have begun to adapt to the treatment temperature.

3. 4. Daily Growth Rate

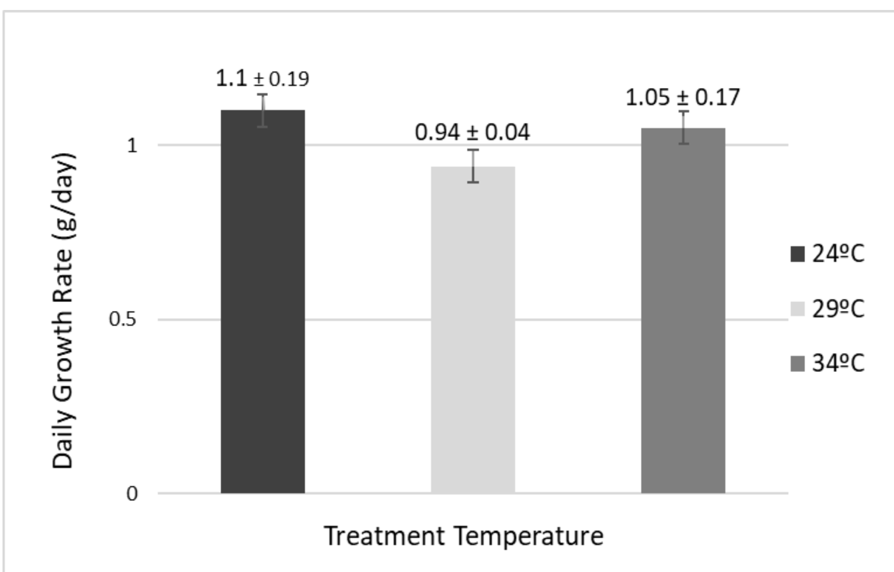

Figure 5. Daily Growth Rate

Based on Figure 5, it shows that the daily growth rate of Common carp Majalaya fingerlings maintained at temperatures of 24 ºC, 29 ºC and 34 ºC respectively by 1.1, 0.94 and 1.05 g/day. Daily weight data are not significantly different among treatments (P>0.05). Its temperature did not affect the daily growth rate of Common carp Majalaya fingerlings.

However, if it is seen from these data that at the 34 ºC treatment temperature, the daily growth rate is greater than the 29 ºC. This occurs because at a temperature of 34 °C the number of fish that survive is less than at the 29 °C so that the lower density allows for better and more optimal fish growth. The daily growth rate depends on several factors, one of which is stocking density which affects the space for fish to move. Many abiotic and biotic component of aquatic ecosystem directly and indirectly influence water quality. Measurement of this component also reflect the dynamics of the living organisms such as metabolic and physiological behaviour of aquatic ecosystem. pH, temperature, and dissolved oxygen have great influence on fish growth (Ahmad et al. 2008); (Ahmad, et al. 2013).

3. 5. Water Quality

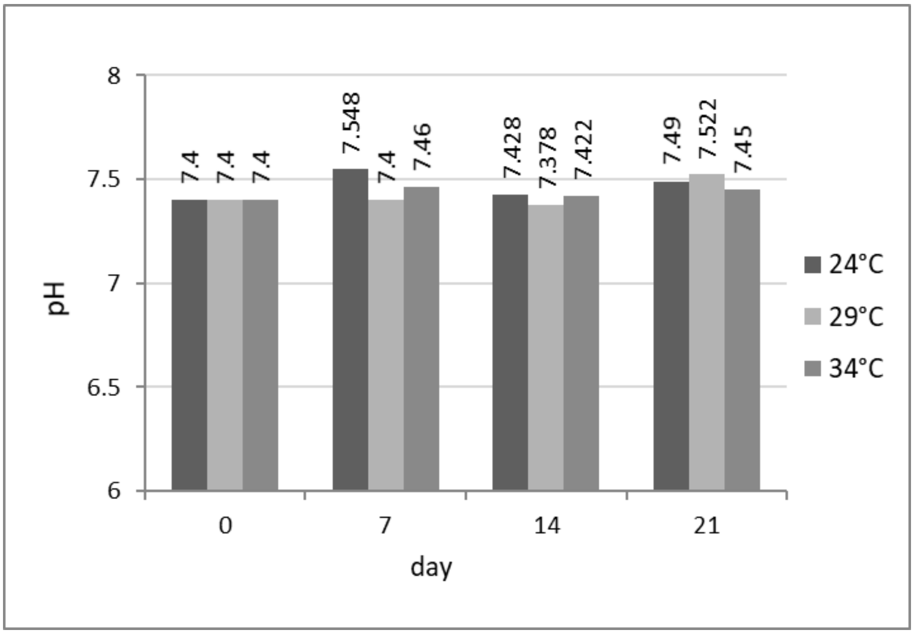

Figure 6. pH

An important factor to ensure good fish production is water pH (Lopes, et al. 2001). The optimum pH range differs among species; According to Heydarnejad (2012) the best range for the survival and growth of carp is pH 7.5-8.0; Common carp achieve optimal survival and growth when exposed to water pH values ranging from 7.5 to 8.0. Based on the pH data in Figure 6, it shows that the pH range during the maintenance time is based on the results of sampling which ranges from 7.38 - 7.55.

The pH value means that it is still within the optimal range of Common carp Majalaya (6.5-8.5) and there is no significant different among the treatments (P>0.05).

Figure 7 shows that the treatment temperature of 24 ºC is with an average DO above 6 mg/l, the treatment temperature of 29 ºC which ranges from 4.14 to 4.5 and the treatment temperature is 34 ºC which is only 3.16 to 3.24 mg/l. According to the Indonesian National Standard, minimum dissolved oxygen for maintenance is 5 mg/l, in this case shows that the treatment with a temperature of 24ºC is the best temperature for DO acquisition because it is still above 5 mg/l and the results (6.14, 6.12, 6.12) are related to the statement from Yanbo & Zirong (2006) dissolved oxygen for common carp is above 6 mg/l. Treatments with temperatures 29 ºC and 34 ºC have an average DO value that is below the minimum rate of dissolved oxygen. It means the fish mortality at the temperature treatment of 29 ºC and 34 ºC were reasonable because DO is an important parameter to support the survival of fish.

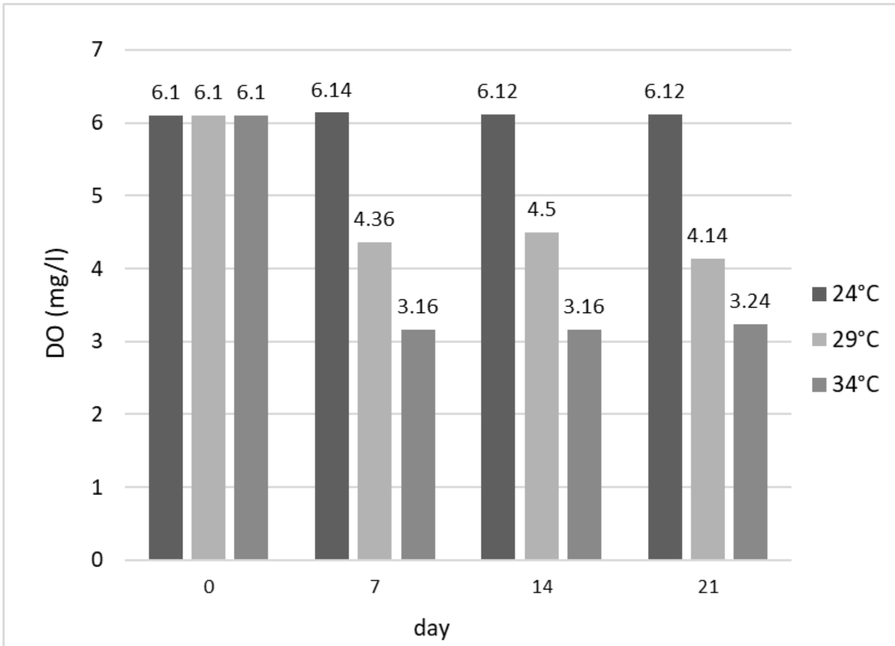

Figure 7. Dissolved Oxygen

4. CONCLUSION

Based on the results of the study, it can be concluded that the treatment temperature of 24 ºC is the best temperature for Common carp Majalaya fingerlings maintenance based on the survival rate value by 100% and the average number of erythrocytes from the 7th day to the 21st day shows a consistent number (2.82, 2.82 and 2.81 × 10^6 cells/mm^3) and it is still within the normal range for the number of common carp erythrocytes (2.98 × 10^6 cells/mm^3); Its leukocytes also show a consistent number (13.73, 13.71 dan 13.88 × 10^4 cells/mm^3) and it is close to the average number of healthy Common carp (12.55 × 10^4 cells/mm^3), both has no significant change so that the fish were in good condition and normal.

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