Hematological analysis of common carp (Cyprinus carpio) using hematology analyzer tools and manual at fish seed center, Pasuruan, East Java

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Abstract. Hematology is one of the branches of science used to determine the condition of fish. In the calculation of blood cells in fish takes a relatively long time that will affect the accuracy of the results of blood cell calculations. Therefore, to overcome the problem, the research on hematology analysis is crucial. This study aimed to determine the efficiency of hematology analyzer tools and hand tally counter and to know the description of differential components of leukocytes, hematocrit, and hemoglobin in Common carp. The method used was the experimental and descriptive method. The results showed that hematology analyzer tools more accurate and efficient than using the hand tally counter. The application of Hematology analyzer tools resulted in a more accurate calculation of erythrocyte cells and leukocyte cells compared to manually using a hand tally counter.

1. Introduction
Common carp is one of the freshwater fish that has significant economic value so that the community widely cultivates carp. Besides being kept in ponds, common carp are also often kept in the fields together with rice plants [1]. One of the problems faced in common carp farming is the disease. Disease attacks can occur due to poor environmental conditions or due to bacterial, fungal, or parasitic infections. Examination of hematology parameters can be used to see the health level of fish [2]. Hematology examination includes an examination of hematocrit values, hemoglobin levels, red blood cell counts, white blood cell counts, and deferential observation of blood [3].

So far, hematology observations are still done directly using a microscope, and calculations are done manually using a hand tally counter. This can cause eye fatigue due to observing small objects in large numbers so that it can affect the accuracy of the calculation results at the time of observation [4]. The method for calculating blood cell counts continues to grow today. Thus, research needs to be done on the differences in the use of Hematology Analyzer Tools and the use of hand tally counters on the results of the calculation of red blood cells and white blood cells in fish.

The purpose of this study was to determine differences in the results of erythrocyte cell counts and leukocyte cells in common carp (C. carpio) using hematology analyzer tools and hand tally counters.
as well as to determine the differential components of leukocytes, hematocrit, and hemoglobin in carp 
(C. carpio).

2. Material and methods
This research was conducted at the Fish Culture Laboratory of the Fish Disease and Health Division
and the Fisheries Product Technology Laboratory of the Fisheries Product Safety Division, Faculty of
Fisheries and Marine Sciences, Brawijaya University Malang and the Fish Seed Management Center,
Pasuruan, East Java in February - March 2018. This research used experimental and descriptive
methods. The experimental method was carried out on test animals for the calculation of erythrocyte
cells and leukocyte cells using the T-test. While the descriptive method was used to see a specific
individual or group phenomena, in this study, it was used for observation of leukocyte diffraction,
hematocrit, and hemoglobin.

2.1. Taking blood samples of common carp
Blood was drawn using a syringe needle containing 3.8% Na citrate as an anti-coagulant. Blood was
drawn from the veins in the lateral line above the ventral fins in the 45˚ position and slowly pulled
until the blood enters the syringe. Then the blood was transferred in a tube for examination.

2.2. The blood analysis
The blood parameters were analyzed and calculated by following the method of Tang et al. [5]. The
detail of analysis was explained elsewhere.

2.3. Use of hematology analyzer tools (use of the software)
This tool has been awarded a patent with IDP000045252 number, dated March 21, 2017. The first step
that must be done is to open the Matlab software. Then the application is out, click open to open the
file that is browsing . and then click run, click add to the path after that the box appears for the process
of processing images. After that, click browse image then find the photo file that will be used to
process the image. Then click crop to cut off the desired cell count area. Then adjust the dynamic
contrast following the original photo image after dynamic contrast, click fill after that, then adjust the
opening again. After that, click the count section, and figure 1 and figure 2 will appear.

2.4. Data analysis
Hematological analysis of Common carp was carried out using experimental and descriptive research.
Erythrocyte and leukocyte cell data obtained by manual calculation using hand tally counter and
hematology analyzer tools are tested using a t-test. Data obtained from descriptive studies are data on
the results of hematocrit, hemoglobin, and differential leukocytes of Common carp.

3. Results and Discussion
3.1. Results of erythrocyte cells and leukocyte cells of common carp
In this study, the process of manually counting the number of erythrocyte cells and leukocyte cells was
done using a hand tally counter commonly done on a laboratory scale, which is directly counted
through a microscope. However, the results were less accurate because of the foresight of the eye,
degree of eye fatigue, and different levels of expertise between laboratory assistants. As for the
calculation process using Hematology analyzer tools, the results were more accurate because it was
done computer-based through image processing of blood cell photos under a microscope.

Processing was done to get the total number of objects that were detected and the total number of
objects that were counted as cells. All detected objects and objects that were counted as cells each
have a roundness level, where the value of roundness was used to distinguish between erythrocyte
cells or leukocyte cells and which were not included as erythrocyte cells or not leukocyte cells. If cells
in the calculated area have a roundness value above 0.75, then it can be said to be an erythrocyte cell,
and if it has a roundness value below 0.75, it does not include erythrocyte cells. A cell object can be
said to be a blood cell if it has a roundness value above 0.75. Figure 1 is the result of processing erythrocyte cells [6]. While Figure 2 is the result of processing leukocyte cells.

![Erythrocyte processing Results](image1)

**Figure 1.** Erythrocyte processing Results from a. Cropping results, b. Dynamic contrast and filling results, c. Results of opening, d. Coloring results, e. Erythrocyte cell object roundness level results.

![Leukocyte processing results](image2)

**Figure 2.** Leukocyte processing results a. Cropping results, b. Dynamic contrast and filling results, c. Results of opening, d. Coloring results, e. Roundness level feature results.

3.2. *Results of common carp (C. carpio) erythrocyte cell calculation*

Based on the results of the study conducted, Table 1 shows the results of the calculation of erythrocyte on common carp (C. carpio) cells.
Table 1. Results of common carp (C. carpio) erythrocyte cells calculation.

| n       | Treatment | Application (A) | Manually (B) | (A-B) |
|---------|-----------|-----------------|--------------|-------|
|         |           |                 |              |       |
| Common carp 1 | 1.100.000 | 1.070.000 | 30.000 |
| Common carp 2 | 1.000.000 | 960.000 | 40.000 |
| Common carp 3 | 1.690.000 | 1.660.000 | 30.000 |
| Common carp 4 | 1.350.000 | 1.300.000 | 50.000 |
| Common carp 5 | 1.730.000 | 1.640.000 | 90.000 |
| Total     | 6.870.000 | 6.630.000 | 240.000 |
| Average   | 1.374.000 | 1.326.000 | 48.000 |
| t-count   | 4.31      |                |              |       |

Table 1 was the result of the calculation of common carp (C. carpio) erythrocyte cells. The average erythrocyte cells in calculations using the application obtained greater results that were 1.374 x 10^6 / mm^3 while for the average calculation, manually obtained results of 1.326 x 10^6 / mm^3. The results of this study showed that common carp erythrocyte cells were still in the normal range. The average normal range of common carp erythrocyte cells is 1.27 - 1.38 x 10^6 / mm^3 [7].

The results of the t-test for common carp erythrocytes (C. carpio) showed that t count was greater than t table 5% or 4.31> 2.77, which means that it was significantly different. The calculations using the software gave significantly different results compared to manuals, or in other words, the calculation of erythrocyte cells using Hematology analyzer tools was more accurate than the hand tally counter.

3.3. Result calculation of common carp leukocyte cells (C. carpio)

Based on the results of the study conducted, Table 2 shows the results of the calculation of leukocyte cells in carp (C. carpio).

Table 2. Results of common carp (C. carpio) leukocyte cell calculation.

| n       | Treatment | Application (A) | Manually (B) | (A-B) |
|---------|-----------|-----------------|--------------|-------|
|         |           |                 |              |       |
| Common carp 1 | 268.850 | 263.000 | 5.850 |
| Common carp 2 | 239.150 | 233.200 | 5.950 |
| Common carp 3 | 268.950 | 259.400 | 9.550 |
| Common carp 4 | 306.150 | 290.700 | 15.450 |
| Common carp 5 | 279.450 | 263.950 | 15.500 |
| Total     | 1.362.550 | 1.310.250 | 52.300 |
| Average   | 272.510 | 262.050 | 10.460 |
| t-count   | 4.87      |                |              |       |

Table 2 was the result of the calculation of common carp (C. carpio) leukocyte cells. The average leukocyte cells in calculations using the application obtained greater results, namely 272,510 cells / mm^3, while the manual calculations obtained results of 262,050 cells / mm^3. The results showed that common carp leukocyte cells were in the normal range. The average normal range of common carp leukocyte cells is 15.6 x 10^3 - 29.1 x 10^3 cells / mm^3 [8].

The results of the t-test for common carp leukocytes (C. carpio) showed that the t count was greater than t table 1% or 4.87> 4.60, which means that the difference was very significant. The calculation
using the application gave significantly different results compared to the manual, or in other words, leukocyte cell calculation using Hematology analyzer tools was more accurate than using a hand tally counter.

3.4. Hematocrit results of common carp (C. carpio)
Based on the results of the study conducted, Table 3 shows the results of the measurement of hematocrit values of all Common Carp (C. carpio) blood samples.

| Common carp | Hematocrit (% | Normal range | Explanation |
|-------------|--------------|--------------|-------------|
| 1           | 31           | 25 - 32      | Normal      |
| 2           | 25           | Normal       |
| 3           | 26           | Normal       |
| 4           | 26           | Normal       |
| 5           | 28           | Normal       |

The increase in hematocrit values is caused by the stress response of fish due to environmental changes, which will later cause swelling of red blood cells due to decreased blood volume. The hematocrit value, the number of erythrocytes, and hemoglobin are related [10]. The percentage of hematocrit reflects the proportion of red blood cells in the blood related to leukocytes and plasma. High hematocrit and lower red blood cells because fish cannot breathe properly and suppress the number of red blood cells [11].

3.5. Results of common carp hemoglobin (C. carpio)
Based on the results of the study conducted, Table 4 shows the results of measurement of the hemoglobin values of all carp samples (C. carpio).

| Common carp | Hemoglobin (g/dl) | Normal range | Explanation |
|-------------|-------------------|--------------|-------------|
| 1           | 6.2               | Normal       |
| 2           | 7.4               | Normal       |
| 3           | 6.6               | 5.9 - 9.09   | Normal      |
| 4           | 6.6               | Normal       |
| 5           | 6.8               | Normal       |

Changes in the value of hemoglobin can also cause the circulatory system to be disrupted, one of which is due to environmental factors. Hematological parameters of each fish are different such as hematocrit, hemoglobin, erythrocytes, and leukocytes. Its caused by environmental conditions, gender, age, origin, genetics, and food [12]. A significant change in hemoglobin concentration was caused by the inability of fish to carry adequate amounts of oxygen into the blood tissue [13].

3.6. Results of common carp differential leukocytes (C. carpio)
Based on the resulted of the study, Table 5 shows the results of the differential leukocytes calculation in a glass object with five replications calculations in one preparation.
Table 5. Average results of carp leukocyte differential (C. carpio).

| Common carp | Limfocyte (%) | Monocyte (%) | Basophil (%) | Eusinophil (%) | Neutrophil (%) |
|-------------|---------------|--------------|--------------|----------------|----------------|
| 1           | 59.6          | 6.6          | 0.2          | 1.6            | 32             |
| 2           | 86            | 0.4          | 0            | 0              | 13.6           |
| 3           | 67.2          | 12.4         | 0.4          | 0              | 20             |
| 4           | 73.6          | 11           | 0            | 0              | 15.4           |
| 5           | 80.2          | 7.6          | 0            | 0              | 12.2           |
| Average     | 73.32         | 7.6          | 0.12         | 0.32           | 18.64          |

Table 5 shows the average yield of carp leukocytes during the study. It can be seen that the average common carp lymphocyte was 73.32%, for an average common carp monocyte was 7.6%, and for an average neutrophil of a common carp was 18.64%. Lymphocytes in carp by 79%, neutrophils by 18%, monocytes by 2%, and eosinophils by 0.65% [14].

Physiological values of differential leukocyte counts are needed to compare and evaluate changes in stressed fish. Stress load can occur due to treatments such as experiments and naturally [15]. Lymphocytes contribute significantly to total white blood cells. Lymphocytes are usually responsible for specific types of immune responses. An Increase in lymphocyte triggers antibody production [16]. Granulocytes in fish blood augmented within 24 hours after stress. Granulocytes in fish blood are usually neutrophils. Reducing the number of granulocytes in the blood may be associated with increased disease resistance [17].

4. Conclusion
The application of Hematology analyzer tools resulted in a more accurate calculation toward erythrocyte and leukocyte cells compared to manually using a hand tally counter. Furthermore, The description of hematocrit, hemoglobin, and differential leukocytes showed that the common carp (C. carpio) model was still in a normal range. Hence, the fish were in a healthy condition.

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