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Hematological Analysis of Nile Tilapia (*Oreochromis niloticus*) and Striped Catfish (*Pangasius hypophthalmus*) using Hematology Analyzer Tool Software at Fish Breeding Center Jojogan, Tuban, East Java

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KEYWORDS

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Abstract Nile tilapia and Striped Catfish can be classified as freshwater fish species that have great cultivation potential. Inadequate cultivation management leads to the occurrence of disease attacks on fish. Hematological examination was applied to determine the level of fish health. This research was aimed to know the difference between erythrocyte cell and leucocyte cell count by using Hematology Analyzer Tool Software (computer based) and hand tally counter (human based), and to know the description of hematocrit component, hemoglobin and leucocytes differential on tilapia fish and Striped Catfish. The methods employed in this research were experimental method and descriptive method. The results showed that the calculation of erythrocyte cells and leucocyte cells by using Hematology Analyzer Tool Software was similar to the calculation by using hand tally counter. It was because the result of t value was smaller than t of the 5% table. Based on the description of the hematocrit component, hemoglobin and leucocyte differentials, it could be said that nile tilapia and Striped Catfish were within the range of abnormalities.

Introduction

The hematological value is closely related to pathological conditions, especially to obtain an image of the health condition of the fish (Zuhrawati, 2014). Hematologic examination is performed by direct blood cell observation through a microscope that requires high concentrations. Small object size, large number, and eyestrain often affect the accuracy of the calculation of blood cell count (Noercholis and Wijaya, 2015).

The method of calculating the number of blood cells is continuously growing up to date and it is a challenge for researchers to continue improving the accuracy of the calculations. Several previous studies have been conducted to provide an alternative calculation of blood cell counts in image by using Hematology Analyzer Tool Software. Hematology Analyzer Tool Software is a computer program that serves to calculate the number of blood cells by processing digital images (Noercholis et al., 2013).

This research was aimed to know the difference between erythrocyte cell and leucocyte cell count of Nile Tilapia (*O. niloticus*) and Striped Catfish (*P. hypophthalmus*) by using Hematology Analyzer Tool Software and hand tally counter. Second, this research was also intended to know the description of hematocrit, hemoglobin, and leucocyte differential components.

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Material and methods

This research was conducted at three different places. First, it was conducted at Fish Disease and Health Division of Fish Cultivation and Laboratory. Second, it took place at Fisheries Safety Division of Fisheries Technology Science Laboratory of Brawijaya University Faculty of Fishery and Marine Science, Malang. Last, it was carried out at Fishing Center of Jojogan, Tuban, East Java.

The methods used in this research were experimental method and descriptive method. The experimental method was performed for the calculation of erythrocytes and leucocytes by using t-test. Then, descriptive method was used for the observation and decomposition of hematocrit, hemoglobin and leucocyte differential. The sample was observed by using a microscope to be calculated manually by using a hand tally counter. It was also documented to be calculated by using the Hematology Analyzer Tool Software modification of Noercholis et al. (2013).

Erythrocyte and Leucocyte Observation

According to Dianti *et al*. (2013), the blood sample was taken by using **erythrocyte** thoma pipette up to 0.5, it was diluted in Hayem Solution until reaching the 101 limits. The blood sample was taken by using **leucocyte** thoma pipette up to 0.5, and then it was diluted by using Turk Solution until reaching the 11 limits. After that, for erythrocyte observation, the fish blood and Hayem Solution were homogenized by shaking it for ± 30 seconds. For Leucocyte observation, the fish blood and Turk Solution were homogenized by shaking it for ±30 seconds Before the sample was being dripped on hemocytometer, the amount of the sample was reduced for 3-4 drops. Next, the hemocytometer was slowly covered with a glass cover to avoid the appearance of bubbles. The formula used to calculate erythrocyte and leucocyte cells were stated below:

$$\text{Number of Erythrocyte} = \sum N \times 10^4$$

Note: N: number of the calculated erythrocyte

$$\text{Number of Leucocyte} = \sum N \times 50$$

Note: N: number of the calculated leucocyte

Leucocytes Differential Observation

The observation of leucocyte differential was done by taking one drop of blood from tube by using syringe, then it was placed on glass object. Next, it was flattened by using the smear method. The smear method was carried out by pulling forward the blood that had been dripped on the glass object using another glass object. After that, the sample was aerated until it was dry on the tray. After being dried, the sample was fixated using methanol, then it was left to dry once again by let it stand on a tray. Furthermore, the dried sample was given Giemsa Stain Solution and it was left up to 15-20 minutes. After 15-20 minutes, the sample was rinsed with aqua distillate. Wait until it was dry for the last time and then observed it using a microscope with 400x magnification (Hidayaturrahmah, 2015).

Hematocrit Calculation

The measurement of hematocrit using a hematocrit capillary pipette was collected by filling a 4/5 parts of hematocrit capillary with blood. Then, both sides of the hole were closed using a Critoseal (wax cover). Next, centrifuge was done within the speed of 12,000 rpm for 4 minutes. After 4 minutes, the centrifuge cover was removed to see the result by reading the hematocrit table scale expressed in percentage (Zuhrawati, 2014).

Hemoglobin Calculation

The measurement of hemoglobin was performed by using Hb Sahli (Sahli's Hemoglobinometer). The blood sample was obtained by using Sahli's pipette up to 20 mm (the line limit of Sahli's pipette) and added in 0.1 N HCl Solution up to number 2 (the yellow line

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boundary). Blood sample was inserted in Sahli's tube and waited until turning into blackish brown. Then, the aqua distillate was added till it corresponded with the color of the two tubes of Hb-Sahli. Furthermore, the hemoglobin value was read in unit g/dl (Simanjuntak, S. B. I. et al., 2018).

Data Analysis

The data of erythrocyte and leucocyte cells of Nile Tilapia and Striped Catfish which were obtained by manual calculation by using hand tally counter and Hematology Analyzer Tool Software were analyzed by pairing t value with t table.

Results and discussion

Results of Erythrocyte Cell and Leucocyte Cell Processing

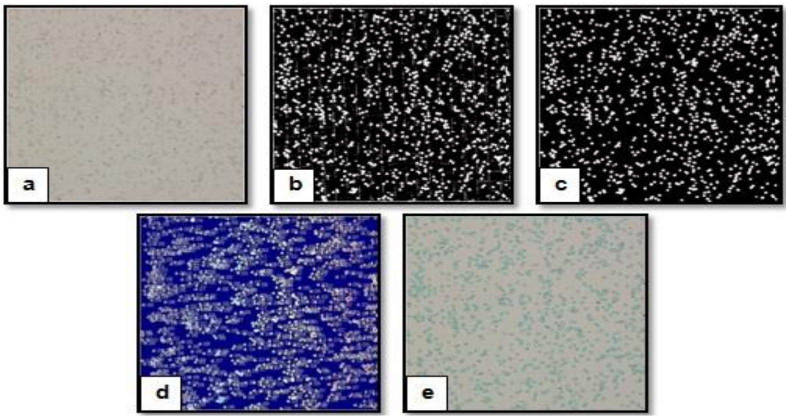

Figure 1. Results of Erythrocyte Processing: a. Cropping Result, b. Dynamic Contrast and Filling Results, c. The Opening Result, d. Roundness Level Feature Result, e. Coloring Result. Step a – e was the process of calculating erythrocytes using fish cell hematology analyzer tool software. Using a microscope with 400x magnification.

The calculation of erythrocytes number was peformed manually by hand tally counter. It was relatively faster and could be directly calculated through a microscope but the results obtained were less accurate, because it required eye care and accuracy when clicking on hand tally counter. As for the main process, it was administered by using Hematology Analyzer Tool Software. This method took a relatively longer time because it should process the image obtained from the microscope. Nevertheless, the result was more accurate because the calculation process was done by using software. It can be seen in Figure 1 the result of erythrocyte cell processing and Figure 2 is the result of leucocyte cell processing.

Processing was done to get the total number of detected objects and total objects counted as cells. All detected objects and objects counted as cells have a roundness rate; the value of this roundness was used to distinguish between erythrocyte or leucocyte cells and the non-erythrocyte cells or non-leucocyte cells. If the cell value that existed within the calculated area was above 0.75, it could be assumed to be an erythrocyte cell and if it had a value below 0.75, it was not the erythrocyte cells. This was in accordance with the research of Noercholis et al. (2013), a cell object could be stated as a blood cell if it has a value of roundness above 0.75.

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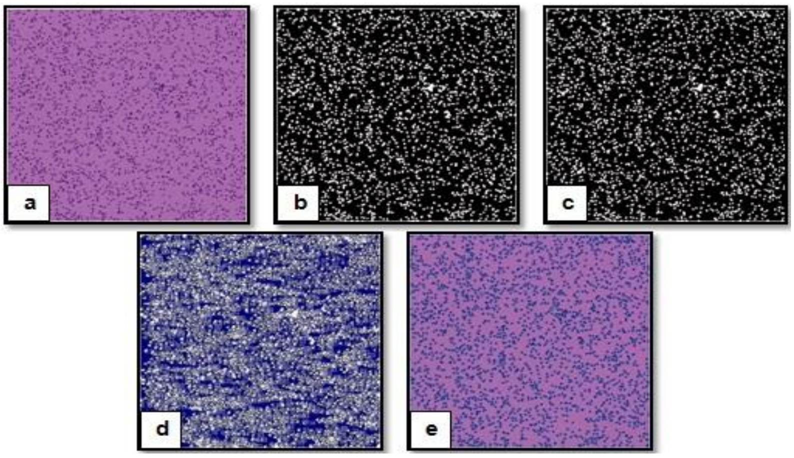

Figure 2. Leucocyte Processing Results: a. Cropping Result, b. Dynamic Contrast and Filling Result, c. The Opening Result, d. Roundness Level Feature Result, e. Coloring Result. Step a – e was the process of calculating Leucocyte using fish cell hematology analyzer tool software. Using a microscope with 400x magnification.

The Erythrocyte Cells of Nile Tilapia (O. niloticus) and Striped Catfish (P. hypophthalmus)

Table 1. Calculation Result of the Erythrocyte Cells of Nile Tilapia (*O. niloticus*) and Striped Catfish (*P. hypophthalmus*)

Research Object	The Nile Tilapia Erythrocyte Cells ($10^{6\ c}$ cell/mm^3)			The Erythrocyte Cells of Striped Catfish (10^6 cell/mm^3)		
	Aplication	Manual	Standard	Aplication	Manual	Standard
Fish 1	1.78	1.63	(Ismail and Mahboub, 2016)	2.87	2.86	(Yaghobi et al., 2014)
Fish 2	0.81	0.81	1.13 – 1.31	3.02	3.13	3.05
Fish 3	1.56	1.72		2.00	2.18	
Fish 4	0.73	0.74	(Osman et al., 2018)	2.72	2.85	(Vhatkar et al., 2013)
Fish 5	0.47	0.41	1.49 – 2.39	0.73	0.85	2.28
Total	5.35	5.31		11.34	11.87	
Average	1.07	1.06		2.26	2.37	

Based on the research results presented in Table 1, it could be seen that the mean of Nile Tilapia's erythrocyte cell using application calculation was 1.07 x 10^6/mm^3 while in manual calculation was 1.06 x 10^6/mm^3 ($P > 0.01$). The average number of erythrocyte cell of the Nile Tilapia was below the range value within literature.

According to Ismail and Mahboub (2016), the average number of erythrocyte cells in Nile Tilapia was 1.13-1.31 x 10^6/mm^3. Meanwhile, according to Osman et al. (2018), the average number of erythrocyte cell was 1.49 - 2.39 x 10^6/mm^3. Many factors could cause the reduction of erythrocyte cells. One of them was caused by polluted aquatic environment. This was supported by research of Osman et al. (2018). It stated that there was a significant reduction in the value of red blood cells, hemoglobin, and hematocrit of tilapia samples taken from polluted environments compared to those which were taken from less polluted environments.

Based on Table 1, the average calculation of Striped Catfish 's erythrocyte cell by using the application calculation, the obtained result was 2.26 x 10^6/mm^3, while the manual calculation showed 2.37 x 10^6/mm^3 ($P > 0.01$). The mean

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results of erythrocyte cell counts were below the range values in the literature.

According to Yaghobi et al. (2014), the number of Striped Catfish erythrocyte cells had a value of 3.05 x $10^6/mm^3$. Meanwhile, according to Vhatkar et al. (2013), the average number of Striped Catfish erythrocyte cells was 2.28 x $10^6/mm^3$. Basically, the number of erythrocytes and hemoglobin were interconnected. If there was a decrease in the number of erythrocyte cells and hemoglobin levels, it would cause the damage of oxygen transportation system of fish metabolism system. In addition, changes in the number of erythrocyte cells could be used also for stress indicators in fish due to the presence of toxins or pollutants in aquatic environments (Siakpere et al., 2009).

Leucocytes Cells of Nile Tilapia (O. niloticus) and Striped Catfish (P. hypophthalmus)

Table 2 presented the calculation of Nile Tilapia's (*O. niloticus*) leucocytes by using application. The obtained result was 274,760 cells/mm^3 while the manual count of Nile Tilapia fish blood sample was 307,250 cells/mm^3 ($P > 0.01$). The average number of Nile Tilapia's leucocyte cells was above the range value in the literature.

According to Guimaraes et al. (2014), the average leucocyte number of Nile Tilapia was 120,000 – 230,000 cells/mm^3. Meanwhile, according to Osman et al. (2018), the total leucocyte number of Nile Tilapia was about 35,670 – 50,650 cells/mm^3. Many factors could cause the escalating of leucocyte cells. One of them was caused by polluted aquatic environment. This was supported by the research conducted by Mazrouh et al. (2015), it explained that a very clear growing number of leucocytes in fish samples collected were caused by pollutants. In line with the opinion of Maftuch et al. (2017), that pollutants increased haemorrhage which resulted in fish organs damage. An intensification in the number of lymphocytes, monocytes, and neutrophils was thought to be the reason for the increasing total number of leucocytes. Another reason that could increase the number of leucocytes was bacterial infection.

Table 2 of Striped Catfish leucocyte cell calculation (*P. hypophthalmus*) by using application obtained result of 119,900 cells/mm^3, while manual calculation reached the number of 194,640 cells/mm^3 ($P > 0.01$). The calculation of leucocytes of Nile Tilapia fish was above the range value in the literature.

Table 2. Calculation Result Leucocyte Cells of Nile Tilapia (*O. niloticus*) and Striped Catfish (*P. hypophthalmus*).

Research Object	The Leucocyte Cells of Nile Tilapia (cell/mm^3)			The Leucocyte Cells of Striped Catfish (cell/mm^3)		
	Application	Manual	Standard	Application	Manual	Standard
Fish 1	350,050	415,950	(Guimaraes *et al*., 2014)	119,900	95,750	(Yaghobi *et al*., 2014)
Fish 2	305,400	382,750	120,000 – 230,000	152,100	94,050	70,000 – 123,750
Fish 3	278,600	296,350		122,000	57,600	
Fish 4	231,950	243,700	(Osman *et al*., 2018)	212,250	266,750	(Pimpimol *et al*., 2012)
Fish 5	207,800	197,500	35,670 – 50,650	368,100	459,050	94,200 – 101,700
Total	1,373,800	1,536,250		974,350	973,200	
Average	274,760	307,250		119,900	194,640	

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According to Yaghobi et al. (2014), the average number of leucocyte cells in Striped Catfish was 70,000 – 123,750 cells/mm^3. In addition, Pimpimol et al. (2012), said that the average mean number of leucocyte cells in Striped Catfish ranged from 94,200 – 101,700 cells/mm^3. Musa et al. (2013) also stated that the growing number of leucocyte cells could be caused by fish's stressful conditions due to infection attacks of either bacteria, fungi, or parasites.

Leucocytes Differential of Nile Tilapia (O. niloticus) and Striped Catfish (P. hypophthalmus)

Table 3. Average Result of Leucocyte Differential Value of Nile Tilapia (*O. niloticus*) and Striped Catfish (*P. hypophthalmus*)

Sample	L (%)	M (%)	B (%)	E (%)	N (%)	Sampel	L (%)	M (%)	B (%)	E (%)	N (%)
N. Tilapia 1	19	26	13.4	6.6	35	Striped Catfish 1	33.6	38.6	1.6	3.8	22.4
N. Tilapia 2	23	23	11.6	6.4	36	Striped Catfish 2	33.6	29.2	2.4	6	28.8
N. Tilapia 3	28	23	14.6	6.4	28	Striped Catfish 3	28.2	28.4	2	11.6	29.8
N. Tilapia 4	36	21	8.2	4.8	30	Striped Catfish 4	22.2	34.8	4.6	7.6	30.8
N. Tilapia 5	32	22	11.4	6.6	28	Striped Catfish 5	29.4	25	8	12.4	25.2
Total	138	115	59.2	30.8	157	Total	147	156	18.6	41.4	137
Average	27.6	23	11.84	6.16	31.4	Average	29.4	31.2	3.72	8.28	27.4

Description: L: Lymphocyte; M: Monocyte; B: Basophils; E: Eosinophils; N: Neutrophils

Table 3 presented that on blood samples of Nile Tilapia, neutrophil cells were more commonly found with an average of 31.4% and eosinophil cells were slightly found with an average of 6.16%. According to Sayed and Moneeb (2015), the average value of lymphocyte cells in the tilapia was 90.89% for the average monocyte cell value of 2%; the mean cell value of neutrophils was 5.67%, and the eosinophil cells' average value was 1.44%. While in the conducted study by Osman et al. (2018), the result of differential leucocyte obtained of lymphocyte cell value was 59.5%; the neutrophil cell value was 24.22%, and the monocyte cell value was 14.47%, and the eosinophil was equal to 1.8%.

Table 3 also showed that in blood samples of Striped Catfish, monocyte cells were more commonly found with an average of 31.2% and basophil cells were slightly found with an average of 3.72%. According to Kumar and Ramulu (2013), the average value of lymphocytes in Striped Catfish was between 68% -72% and the average value of neutrophils in Striped Catfish was 23%-8%. Furthermore, the mean value of monocytes in Striped Catfish was 2%-3%, and the average eosinophil value in Irisdecent Shark was 1%-3%. Lymphocytes were the most common cells found in fish leukocytes because lymphocytes served as the body's defense system against infection. Changes in the number of leukocyte cells could be caused due to the stress resulted in the declining of the fish's immune system which made it susceptible to disease attack (Ariweriokuma *et al*., 2016).

Hematocrit of Nile Tilapia (O. niloticus) and Striped Catfish (P. hypophthalmus)

The results of the research displayed on Table 4 showed that the hematocrit value of Nile Tilapia was below the normal range. According to Osman *et al*. (2018), the hematocrit value of Nile Tilapia ranged from 20.49 - 38.76%. Significant reductions occurred in the value of red blood cells, hemoglobin, and hematocrit of tilapia samples taken from polluted environments compared with those taken from less polluted environments.

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The results of Table 4 also portrayed that the hematocrit values of Striped Catfish were below the normal range. According to Yaghobi *et al*. (2013), the hematocrit value of Striped Catfish ranged from 37 - 40%. According to Tamamdusturi *et al*. (2016), the decreased number of hematocrits, hemoglobin and erythrocytes could be caused by an infection that may be resulted in the changing of red blood cells to become lysis.

Table 4. Hematocrit (Ht) dan Hemoglobin (Hb) Results of Nile Tilapia (*O. niloticus*) and Striped Catfish (*P. hypophthalmus*)

The Hematokrit and Hemoglobin of Nile Tilapia (*O. niloticus*)					The Hematokrit and Hemoglobin of Striped Catfish (*P. hypophthalmus*)			
Sample	**Ht (%)**	**Standart Ht**	**Hb (g/dl)**	**Standart Hb**	**Ht (%)**	**Standart Ht**	**Hb (g/dl)**	**Standart Hb**
Fish 1	21		7		44		8.6	
Fish 2	15	(Osman *et al*., 2018)	6	(Osman *et al*., 2018)	28	(Yaghobi *et al*., 2014)	10	(Yaghobi *et al*., 2014)
Fish 3	18	20.49 – 38.76	6.4	7 – 11.55	29	37 – 40	6	12.58 – 13.98
Fish 4	16		5.8		38		6.8	
Fish 5	15		5.4		32		8.8	
Total	85		30.6		171		40.2	
Average	17		6.12		34.2		8.04	

Nile Tilapia (O. niloticus) and Striped Catfish (P. hypophthalmus) Hemoglobin

Based on the results of research at Table 4, Tilapia's hemoglobin values were below the normal range. According to Osman *et al.* (2018) the value of tilapia hemoglobin ranged from 7 - 11.55 g/dl. According to Summarwar (2012), there was a remarkable decrease number in red blood cells, hemoglobin, and hematocrit from contaminated environments compared to unstained environments.

The value of Striped Catfish 's hemoglobin at Table 4 was being below the normal range. According to Yaghobi *et al.* (2013), hemoglobin values ranged from 12.58 to 13.98 g/dl. In addition, Daneshvar *et al.* (2012), stated that the main function of hemoglobin was to transport oxygen and was one of the parameters of fish health. The decrease in hemoglobin concentration could be caused due to fish infections within stressful conditions. Stress that occurred could be caused by the uncontrolled environmental conditions.

Water Quality Parameters

The average water quality parameters measured on Nile Tilapia's fish ponds: pH 7.6; DO 2.32 mg/l; Temperature 30 °C. The above results showed that the value of DO (Dissolved Oxygen) was below the optimal range, while the pH and temperature were in the optimal range. According to Azim and Little (2008), water quality for Nile Tilapia was 6.7 of pH value within the range of 5 – 8.5; DO value was 6 mg/l within the range of 3 – 7.5 and for the temperature was 28 °C within the range 26 - 30 °C.

The average water quality parameters measured in Striped Catfish 's ponds: pH 7.4; DO 3.69 mg/l; temperature 31 °C. The water quality parameters for cultivation of Striped Catfish indicated that the pH value and temperature were above the optimal range, but it was still within the acceptable range. However, the value of DO was in the optimal range. According to a study conducted by Hekimoglu *et al.* (2014), the water quality range during the study showed a water pH of 6.52 - 7.1, dissolved

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oxygen 2.59 - 4.24 mg/l and a temperature of 28.1 - 29.9 °C.

Conclusion

Statistically, the results of erythrocyte cell and leucocyte cell count in Nile Tilapia and Striped Catfish by using Hematology Analyzer Tool Software were similar to manual calculation by using hand tally counter. Therefore, it could be concluded that the researcher's skill in calculating the number of erythrocytes and leucocytes was close to perfection.

Fish health level could be referred to the hematological parameter values including the number of erythrocytes, the number of leukocytes, hematocrit, hemoglobin, and leukocyte differential. Based on the value of hematological parameters, the Nile Tilapia and Striped Catfish observed in this study were in unhealthy conditions.

Suggestion

In accordance to the results of the research, the researchers suggest to use Hematology Analyzer Tool Software for the calculation process of fish erythrocytes cells and leukocyte cells.

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