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The erythrocyte and leucocyte profile of saline tilapia (*Oreochromis Niloticus*) in a cultivation system with nanobubbles

Gunanti M[1]*, P D Wulansari[1] and K Kinzella[2]

[1] Department of Fish and Aquaculture Health Management, Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya 60115, Indonesia
[2] Aquaculture Study Program, Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya 60115, Indonesia

*Corresponding author: mahasritot@gmail.com

Abstract. Saline tilapia has a high tolerance when it comes to growing and developing in water salinity of 15 - 25 ppt. The used method was an experiment. The highest level of erythrocytes was found in the 30th day nanobubble (33.63×104 cells/mm^3) and the lowest was on the 0th day of using the aerator (8.25×104 cells/mm^3). The highest level of leukocytes was found on the 30th day of the nanobubbles ($45,175$ cells/mm^3) and the lowest was on the 0th day of the aerator ($15,375$ cells/mm^3). The highest level of basophils was found on the 30th day of the nanobubbles (5.75%) and the lowest was on the 10th day of the aerator (2.38%). The highest level of eosinophils was on the 0th day (4.75%) and the lowest was on 30th day nanobubble (3.38%). The highest level of neutrophils was found on the 10th day (68%) and the lowest level was found on 0 day with the aerator (32.13%). The highest level of lymphocytes was found on the 20th day (55.25%) and the lowest was found on the 30th day with the aerator (18.13%). The highest level of monocytes was found on the 20th day (9.625%) and the lowest was found on the 30th day with the aerator (7.5%). This showed that the cultivation system using nanobubbles affected the number of erythrocytes and leukocytes in the saline tilapia.

1. Introduction

Saline tilapia are a kind of tilapia fish that have a high tolerance ability when it comes to growing and developing in a water salinity of 15 - 25 promil [1]. This makes tilapia in saline different from other types of tilapia because it can survive in high salinity. The success of saline tilapia cultivation depends on several factors including feeding and the water quality [2]. The condition of the fish's aquatic environment is very influential on its survival as besides salinity, the water quality parameter that also needs to be considered is the content of the dissolved oxygen in the cultivation media.

Oxygen also has an important role in the circulatory system of fish. After oxygen enters the gills, it will be absorbed by the blood. Blood that contains oxygen will flow through the arteries to be carried throughout the body. All of these things are the task of the erythrocytes in the blood; erythrocytes are very important because in erythrocytes, there is a substance called hemoglobin which plays a role in binding the oxygen from the environment and carryng it throughout the body to where it needs it. Low levels of erythrocytes will cause the fish to be unable to take oxygen from the water and consequently, the fish will experience anoxia (a lack of oxygen).

Leukocytes in the blood play a role as a component of the body's defenses when the fish is under attack from disease [3]. Blood tests were conducted to determine the pattern of increased immune response by calculating the total leukocytes, differential leukocytes and erythrocytes in the blood [4]. According to [5], he said that high amounts of erythrocytes support more oxygen absorption to better fulfill the oxygen demand to maintain life. Meanwhile, according to [6], low oxygen levels stimulate the formation of new red blood cells in the blood and cause an increase in the number of erythrocytes. Whereas in leukocytes, low oxygen will increase the number of leukocytes in the fish [7].

An effort undertaken to overcome and reduce the occurrence of stress due to aquatic environmental conditions that do not support the fish's optimal condition is nanobubble oxygen technology [8]. Nanobubbles are a technology that can increase the level of dissolved oxygen in aquatic water so then the oxygen in the water is better maintained. Oxygen levels that are well-maintained will also increase the presence of oxygen which can help the metabolic process. The metabolic process requires oxygen to burn nutrients in the catabolic process. Besides that, the oxidation of the food ingredients requires known amounts to produce a certain amount of energy [9]. Nanobubbles have the advantage of the oxygen produced being able to be dissolved in water for a long time. This means that it can save more energy and where the oxygen produced is more stable [8].

2. Research method

2.1 Materials and tools

The used materials in this study were saline tilapia (*Oreochromis niloticus*) with a size of 7 - 10 cm, water with a salinity of 18 - 20 ppt taken from the Instalasi Budidaya Air Payau Lamongan (IBAP), saline fish blood, 2% EDTA, giemsa 20%, hayem solution, turk, methanol and distilled water. The used tools in this research included 8 maintenance boxes measuring 8 cm x 36 cm x 30 cm, nanobubble generators, aerators, aeration hoses, aeration stones, digital scales, DO meters, reservoir tanks, basins, refractometers, syringes, microtube, object glass, staining glass, tweezers, microscope, hemocytometer, thoma pipette, and an object glass.

2.2 Method and research design

This study used an experimental method that was carried out in the Laboratory of Education of the Faculty of Fisheries and Marine in Airlangga University between August and October 2017. The used method was an experiment with a completely randomized design factorial, which consisted of factor A is the use of an aerator and nanobubbles and factor B being the maintenance time, which was over the 0th, 10th, 20th and 30th days respectively. For the aerator treatment, there were four maintenance containers which were the same as nanobubbles. Each maintenance container was filled with 25 liters of water within which was 25 saline tilapia. What was observed in this study was the effect of nanobubble use on the number of erythrocytes and leukocytes in the tilapia saline.

The use of nanobubbles as a source of oxygen in cultivation will make the blood more rich in oxygen [8]. The oxygen will be transported by the hemoglobin in the erythrocytes and circulated throughout the body. It also affects the fish's leukocytes, as the body's defense system will also increase if the whole body gets the appropriate level of oxygen. A low number of erythrocytes shows that the fish have an infection [10], while the number of leukocytes and differential leukocytes can be used as indicators of certain infectious diseases that occur in fish [11].

The calculation of the number of erythrocytes can be done by using the following formula [12]:

$$\text{The number of erythrocytes} = \text{the number of leukocyte cells counts} \times 10^4 \text{ cells / mm}^3 \qquad (1)$$

The calculation of the number of leukocytes can be done by using the following formula [12]:

$$\text{Leukocyte count} = \text{the number of leukocyte cells counts} \times 50 \text{ cells / mm}^3 \qquad (2)$$

The calculation of the differential amount of leukocytes can be done by using the followng formula [13]:

% Lymphocytes$=\dfrac{Lymphocytes}{100}$ x 100%   (3)

% Monocytes $=\dfrac{Monocytes}{100}$ x 100%   (4)

% Neutrophils $=\dfrac{Neutrophils}{100}$ x 100%   (5)

% Eosinophils $=\dfrac{Eosinophils}{100}$ x 100%   (6)

% Basophils $=\dfrac{Basophils}{100}$ x 100%   (7)

3. Research and discussion

The calculation result of erythrocytes in saline tilapia showed there to be a significant difference between the nanobubble treatment and that with the aerator ($p < 0.05$). The number of saline tilapia erythrocytes in the nanobubble treatment increased, while the number in the aerator treatment both increased and decreased.

Table 1. The average calculation of the saline tilapia's erythrocytes during the maintenance period

Treatment	Erythrocytes count (cell/mm^3)
A1B1	$9,38 \times 10^{4cd} \pm 1,37$
A1B2	$19,38 \times 10^{4b} \pm 2,28$
A1B3	$31,75 \times 10^{4a} \pm 1,32$
A1B4	$33,63 \times 10^{4a} \pm 2,17$
A2B1	$8,25 \times 10^{4d} \pm 0,95$
A2B2	$8,5 \times 10^{4d} \pm 0,20$
A2B3	$12,13 \times 10^{4c} \pm 4,02$
A2B4	$11,63 \times 10^{4cd} \pm 1,70$

[a]Different superscripts in the different columns and rows show there to be significant differences ($p < 0.05$)
[b]Information:
[c]A1   : Nanobubble treatment
[d]A2   : Aerator treatment
[e]B 1-4   : Data retrieval from 0, 10th , 20th and 30th days.

The calculation results of the number of leukocytes in the saline tilapia showed a significant difference between the nanobubble treatment and that with the aerator ($p < 0.05$). The number of leukocytes in the tilapia saline in the treatment with nanobubbles and an aerator both increased but in different amounts.

Table 2. The average calculation of saline tilapia leukocytes during the maintenance period

Treatment	Leukocytes count (cell/mm^3)
A1B1	$14.743,75^{f} \pm 1.878,53$
A1B2	$2.9631,25^{c} \pm 671,24$
A1B3	$33.562,5^{b} \pm 1.065,07$
A1B4	$45.175^{a} \pm 2.587,55$
A2B1	$15.375^{ef} \pm 2.687,31$
A2B2	$29.631,25^{ef} \pm 1.474,08$
A2B3	$33.562,5^{de} \pm 544,10$
A2B4	$45.175^{d} \pm 562,5$

[a]Different superscripts in the different columns and rows show there to be significant differences (p <0.05)
[b]Information:
[c]A1   : Nanobubble treatment
[d]A2   : Aerator treatment
[e]B 1-4   : Data retrieval from 0, 10th, 20th and 30th days.

The calculation results of basophil concerning the saline tilapia showed there to be a significant difference between the nanobubble treatment and that with an aerator (p <0.05). The number of basophils in the tilapia saline in the treatment with nanobubbles experienced an increase while the aerator treatment experienced both increases and decreases.

Table 3. The average calculation of the saline tilapia's basophil during the maintenance period

Treatment	Basophil count (%)
A1B1	$3,25^{bcd} \pm 0,65$
A1B2	$4,075^{bc} \pm 1,43$
A1B3	$3,25^{bcd} \pm 0,87$
A1B4	$5,75^{a} \pm 0,96$
A2B1	$4,5^{ab} \pm 1,08$
A2B2	$2,38^{d} \pm 0,63$
A2B3	$3,38^{bcd} \pm 0,63$
A2B4	$2,63^{cd} \pm 1,03$

[a]Different superscripts in the different columns and rows show there to be significant differences (p <0.05)
[b]Information:
[c]A1   : Nanobubble treatment
[d]A2   : Aerator treatment
[e]B 1-4   : Data retrieval from 0, 10th , 20th and 30th days.

The calculation result of the eosinophils in the saline tilapia showed that there were no significant differences between the nanobubbles and aerator treatments (p> 0.05). The number of basophils in the tilapia saline in the treatment with nanobubbles decreased while the aerator treatment experienced both increases and decreases.

Table 4. The average calculation of saline tilapia eosinophils during the maintenance period

Treatment	Eosinophils count (%)
A1B1	$4,75^{a} \pm 2,398$
A1B2	$4,63^{a} \pm 1,493$
A1B3	$4,38^{a} \pm 1,315$
A1B4	$3,38^{a} \pm 1,181$
A2B1	$3,5^{a} \pm 1,581$
A2B2	$3,625^{a} \pm 0,8530$
A2B3	$4,75^{a} \pm 0,645$
A2B4	$4,125^{a} \pm 1,25$

[a]Different superscripts in the different columns and rows show there to be significant differences (p <0.05)
[b]Information:
[c]A1   : Nanobubble treatment
[d]A2   : Aerator treatment
[e]B 1-4   : Data retrieval from 0, 10th, 20th and 30th days.

The calculation results of the neutrophil count in the tilapia saline showed there to be a significant difference between the nanobubble and aerator treatments ($p < 0.05$). The neutrophil number in the saline tilapia in the treatment with nanobubbles increased while the aerator treatment both increased and decreased.

Table 5. The average calculation of saline tilapia neutrophil's during the maintenance period

Treatment	Neutrophil count (%)
A1B1	$45^c \pm 3,34$
A1B2	$42,38^c \pm 5,65$
A1B3	$42,38^c \pm 5,65$
A1B4	$48^c \pm 4,74$
A2B1	$32,13^d \pm 5,27$
A2B2	$68^a \pm 4,67$
A2B3	$55,25^b \pm 3,28$
A2B4	$67,63^a \pm 2,06$

[a]Different superscripts in the different columns and rows show there to be significant differences ($p < 0.05$)
[b]Information:
[c]A1   : Nanobubble treatment
[d]A2   : Aerator treatment
[e]B 1-4 : Data retrieval from 0, 10th, 20th and 30th days.

The calculation results of the lymphocyte count in the saline tilapia showed there to be a significant difference between the nanobubble treatment and the aerator treatment ($p < 0.05$). The lymphocyte count in the saline tilapia in the nanobubble treatment experienced an increase and decrease like in the aerator treatment.

Table 6. The average calculation of the saline tilapia lymphocyte's during the maintenance period

Treatment	Lymphocyte count (%)
A1B1	$44,25^b \pm 6.357$
A1B2	$33^c \pm 4,339$
A1B3	$41^b \pm 4,339$
A1B4	$29,38^c \pm 3,837$
A2B1	$43,13^b \pm 7,157$
A2B2	$19.13^d \pm 3,350$
A2B3	$55,25^a \pm 3,279$
A2B4	$18,13^d \pm 3,473$

[a]Different superscripts in the different columns and rows show there to be significant differences ($p < 0.05$)
[b]Information:
[c]A1   : Nanobubble treatment
[d]A2   : Aerator treatment
[e]B 1-4 : Data retrieval from 0, 10th, 20th and 30th days.

The calculation result of the monocyte number in the saline tilapia showed there to be a significant difference between the nanobubble treatment and aerator treatment ($p < 0.05$). The monocyte count of the saline tilapia in the nanobubble treatment experienced an increase and decrease as well as in the aerator treatment.

Table 7. The average calculation of the saline tilapia monocytes during the maintenance period

Treatment	Monocyte count (%)
A1B1	$13,38^a\pm4,001$
A1B2	$13,25^a\pm4,406$
A1B3	$9^{ab}\pm1,871$
A1B4	$13,5^a\pm2$
A2B1	$13,75^a\pm4,734$
A2B2	$6,875^b\pm1,652$
A2B3	$9,625^{ab}\pm0,854$
A2B4	$7,5^b\pm2,082$

[a]Different superscripts in the different columns and rows show there to be significant differences (p <0.05)

[b]Information:

[c]A1   : Nanobubbletreatment

[d]A2   : Aerator treatment

[e]B 1-4   : Data retrieval from 0, 10th, 20th and 30th days.

The calculation of the erythrocyte level in the saline fish treated with the nanobubbles was always increasing but still within the normal range which was 20,000 - 3,000,000 cells / mm^3[14]. The increase also occured in the number of leukocytes of the saline tilapia count within the nanobubble treatment, but they were still within the normal leukocyte limit of 20,000 - 150,000 cells / mm^3[15]. The increasing number of saline tilapia erythrocytes within the nanobubble treatment was still within normal limits and so this indicates that the fish was not in a state of stress. According to [16], a high number of erythrocytes beyond the normal limit indicates that the fish is in a state of stress. According to [8], the nanobubble systems produce oxygen that can dissolve over a long period time; therefore it can fulfil the needs of the organisms and decompose organic matter. The breakdown of organic material produces a comfortable environment so then the fish does not become stressed. While the increase in the number of leukocytes in the saline tilapia under the nanobubble treatment is within the normal range of leukocytes, it does not indicate that the fish is in a state of pain. According to [11], diseased fish will produce many leukocytes to phagocytize the bacteria and synthesize antibodies.

The differential leukocyte count in the saline tilapia is divided into two forms, namely granulocytes and agranulocytes. Granulocytes consist of white blood cells that contain specific grains in the cytoplasm so then the neutrophils, eosinophils and basophils can be distinguished [17]. Agranulocytes consist of lymphocytes and monocytes. Lymphocytes are small blood cells with large nuclei (occupying the largest part of the cells) that are not granular and that are surrounded by a small amount of cytoplasm [18]. The calculation of the differential leukocytes in saline tilapia with the nanobubble treatment both increased and decreased. The increase in the differential leukocytes can indicate the body's resistance response to disease-causing agents. In basophils, if there are a large number of basophils then it indicates that the fish are infected with fungi while in eosinophils, if there is a large amount, then it indicates that the fish are infected with parasites [19]. As was the case with the number of basophils on the 30th day, they also increased from day 0, as documented on the 10th, and 20th days. This indicated the presence of fungi in fish, while the number of eosinophils decreased compared to the days 0, 10 and 20. This indicates that the parasite attacks were not as high as at the beginning of the maintenance period. In relation to the neutrophils, the increase, according to [20], is related to the main function of the neutrophils, namely the destruction of foreign material through the process of phagocytosis [20]. This increase indicates that the neutrophils are carrying out their function, which is acting against any foreign objects or infections that are in the fish.

While in lymphocytes there was a decrease, this was following the fact that if there is a low lymphocyte count in the blood that is n circulation, then this will be balanced with a high number of neutrophils and vice versa [21]. The increasing number of monocytes indicates that monocytes are needed to produce phagocytes due to the infection that enters the body to stimulate monocyte

production; this is what happened on the 30th day of maintenance. Besides, the increase that occurs in the differential leukocytes of saline tilapia was maintained by the nanobubble treatment because in nanobubble systems, the oxygen can last for long so then it is better utilized for the biological activities of the organism. According to Chiba and Takahashi, nanobubble systems produce oxygen that can dissolve over a long period so then it can fulfill the needs of the organisms and decompose organic matter [8].

4. Conclusion

Cultivation systems with nanobubbles affect the total number of erythrocytes and leukocytes in saline tilapia (Oreochromis niloticus).The cultivation system with nanobubbles affected the differential leukocytes of the saline tilapia (*Oreochromis niloticus*).

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