Physiological Stress Response of Differences in Stocking Density to Hematological Parameters of Red Tilapia (Oreochromis sp.) on Budikdamber System

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Abstract

The fish farming program in buckets (budikdamber) is an environmentally friendly fish farming technique for ecosystems between the development of fish and vegetables by using buckets as a container for fish development and water for development media. Optimization of fish farming with high stocking densities accompanied by high feeding will cause accumulation of organic matter in culture containers which will worsen the quality of the rearing water which will ultimately have an impact on the physiological and hematological conditions of fish. Hematology is a scientific discipline that studies the components of blood cells and the functional disorders of these cells. Under stress conditions, the secondary response will cause changes in the hematological condition of the fish. The purpose of this study was to determine the stress response to changes in hematology from differences in stocking density of red tilapia (Oreochromis sp.) in the Budikdamber system. The experimental design used a factorial complete randomized design with density treatment (A (2 fish/10L), B (4 fish/10L), C (6 fish/10L), D(8 fish/10L) and system (budikdamber with water spinach (a) and without water spinach (b). This study lasted for 4 weeks. Erythrocytes in all treatments experienced their highest point and peak of stress in the 2nd week. Erythrocytes decreased which is suspected that the fish had adapted and recovered to initial hemostasis in the 3rd and 4th week. The best erythrocytes were the Ab treatment (2 fish/10L without water spinach) namely 1,616,666 cells/mm3. Leukocytes in all treatments experienced the highest point and peak stress in the 2nd week. Leukocytes decreased in the 3rd and 4th week which were thought to have adapted and recovered to initial hemostasis. The best leukocytes were treatment Aa (stocking density of 2 fish/10L with water spinach) which had a leukocyte value of 55,266.67 cells/mm3. Hematocrit in all treatments had the highest point and peak stress at week 3. The hematocrit in all treatments decreased except for treatment Aa which experienced a decrease in which it was suspected that the fish had adapted and recovered to initial hemostasis in the 4th week. The best hematocrit was Aa (stocking density of 2 fish/10L with water spinach) which had a hematocrit value of 21.67%. Hemoglobin in all treatments experienced its highest point and peak of stress in the 3rd week. Hemoglobin in all treatments decreased except for treatment Aa in the 4th week which presumably the fish had adapted and recovered to initial hemostasis. The best hemoglobin treatment was Aa (stocking density of 2 fish/10L with water spinach) which had a hemoglobin value of 12 g/dL.

Keywords: *Budikdamber, Stocking Density, Stress, Hematology*.

INTRODUCTION

The fish farming program in buckets is an environmentally friendly fish farming technique for ecosystems between fish and vegetable development by using buckets as a container for fish development and water for development media. The application of fish farming technology in buckets or commonly called "budikdamber" can be a potential solution for aquaculture on narrow land with more efficient use of water, easy for people to do in their own homes with relatively small capital and finally able to meet the nutritional needs of the community . In addition to meeting the needs for animal protein obtained from fish, this development method that adheres to the Budikdamber system also provides vegetables for consumption (Sarah & Pramulya, 2021). Budikdamber can be used to cultivate a variety of freshwater fish such as catfish, tilapia, carp, catfish and sepat fish. The plant that is usually used is water spinach.

The red tilapia which is currently widely developed in Indonesia is a tetrahybrid tilapia which is the result of crossing four different species of the genus Oreochromis, namely Oreochromis mossambicus (Mujair), Oreochromis niloticus (tilapia), Oreochromis hornorum, and Oreochromis aureus (Sucipto & Prihartono, 2007). Water spinach is a plant that can grow almost anywhere in the tropics, from the lowlands to the highlands, especially open land exposed to direct sunlight (Nugroho & Sutrisno, 2008).

Optimization of fish farming with high stocking densities accompanied by high feeding will cause accumulation of organic matter in the culture containers. An increase in stocking density that exceeds the carrying capacity will cause a decrease in growth rate (Arianto et al., 2019). In addition, an increase in stocking density that is very high and even exceeds the tolerance limit can adversely affect the health and physiology of fish leading to stress.

Stress is a number of physiological responses from the body that occur when fish try to maintain homeostasis. According to Haqqawly et al., (2013) stated that under stressful conditions fish will experience a reallocation of basal metabolic energy from activities in the form of growth and reproduction, in which energy will be used to improve homeostasis. In stressful conditions, the body will provide physiological responses which are divided into three responses, namely primary, secondary, and tertiary responses. In the primary phase, a stress stimulant occurs which stimulates the hypothalamus to secrete CRF (Corticoid Releasing Factor) by the hypothalamus paraventricular nucleus which will cause the release of the hormone cortisol (Ismail, 1994). At the same time, the receptor organ will receive information that will be conveyed to the brain in the hypothalamus, then the chromaffin cells will secrete catecholamine hormones. This hormone will suppress the secretion of the hormone insulin which functions to help supply glucose into the cells, thus causing the level of glucose that enters the blood to increase. Fish will give a secondary response in the form of changes in blood biochemistry, tissue histology and blood picture (hematology) (Prihadi et al., 2017). Furthermore, according to (Barton, 2002), stressed fish will show a tertiary response, namely systemic changes that cause a decrease in appetite, causing the body's defense system to decrease, unstable growth, and even death.

Hematology is a scientific discipline that studies the components of blood cells and the functional disorders of these cells. Under stress conditions, the secondary response will cause changes in the hematological condition of the fish. Hematology is also used as a parameter to see and know abnormalities that occur in fish, whether they occur due to disease or due to environmental conditions (Affandi, 2002). Fish blood is composed of plasma fluid and blood cells consisting of red blood cells (erythrocytes), white blood cells (leukocytes), and blood platelets (thrombocytes). Stressed fish will experience changes in hematocrit values, hemoglobin levels, red blood cell counts and white blood cell counts.

Total erythrocytes that are too high indicate the fish is in a state of stress so that the fish produce new red blood cells (Arlanda et al., 2018). In line with the opinion of Fauzan et al., (2017) which stated that a high number of erythrocytes indicated that the fish was in a state of stress, while a low number of erythrocytes was an indicator of anemia. According to Dianti et al (2013), the number of erythrocytes in tilapia generally ranges from 1.05 – 3.0 x 106 cells/mm3.

An increase in leukocytes indicates an increase in the number of antibodies. More leukocytes are produced when the body is sick. Leukocytes play a key role in the immune system and become especially important under stressful conditions (Ni et al., 2014). Environmental incompatibility and the entry of foreign compounds into the body cause fish to experience stress, so that the total leukocytes in the fish's body increase. Normal total leukocytes in tilapia range from 2x104 – 15x104 cells/mm3 (Lagler et al., 1977).

An increase in the hematocrit value in fish is caused by unsuitable environmental conditions, the large number of compounds or foreign substances that enter the fish's body resulting in contaminants, vitamin deficiencies, disease and other problems in the fish's body which can cause stress in fish resulting in the production of lots of red blood cells. . According to Royan et al (2014), the value of hematocrit levels in tilapia ranges from 21.00% - 22.67%.

Hemoglobin has an important role in transporting the main gas oxygen from the gills which is pumped by the heart to all cells and organs of the body. The hemoglobin value which is in the normal range indicates that there is sufficient oxygen bound in the blood so that it describes the health condition of the fish in good condition. High levels of hemoglobin in the blood indicate fish in a state of stress (Yuni et al., 2019). According to Wedemeyer and Yasutake (1997), normal hemoglobin levels in tilapia range from 10 to 11.1 g/dL. If the value of hemoglobin in the blood is less than 5 g/dL, it indicates low levels of hemoglobin in the blood, causing the metabolic rate to decrease and the energy produced to be low.

Based on the explanation above, it is necessary to carry out further research regarding the stress response to hematological changes from differences in stocking density of red tilapia (Oreochromis sp) in the Budikdamber system.

Method

Location and Time of Research

This research was conducted for 28 days (4 weeks) in January 2023 at the fish reproduction laboratory, Faculty of Fisheries and Marine Sciences, Brawijaya University, Malang.

Figure 1. Laboratory of fish reproduction, Faculty of Fisheries and Marine Sciences, University of Brawijaya, Malang

Tools and Materials

The tools used in this study were water spinach seedling tools (rockwoll, tweezers, trays), 16 liters capacity buckets, used aqua cups, wire, paranet, effendorf tube, thoma leukocyte pipette, microcentrifuge, haemocytometer, microscope, and hematocrit reader. The materials used in this study were water spinach seeds, fresh water, 6 cm red tilapia, PF1000 feed, hayem solution, turk solution, ETDA, and blood samples.

Research Design

The experimental design used a factorial complete randomized design with density treatment (A (2 individuals/10L), B (4 individuals/10L), C (6 individuals/10L), D (8 individuals/10L) and system (budikdamber with water spinach (a) and without water spinach (b)) The experimental layout used in this study can be seen in Table 1.

Table 1. Research Layout

Density	System	Deuteronomy		
		1	2	3
A (2 fish/10L)	With Water spinach (a)	1Aa	2Aa	3Aa
	No Water Spinach (b)	1Ab	2Ab	3Ab
B (4 fish /10L)	With Water spinach (a)	1Ba	2Ba	3Ba
	No Water Spinach (b)	1Bb	2Bb	3Bb
C (6 fish /10L)	With Water spinach (a)	1Ca	2Ca	3Ca
	No Water Spinach (b)	1Cb	2Cb	3Cb
D (8 fish/10L)	With Water spinach (a)	1Da	2Da	3Da
	No Water Spinach (b)	1Db	2Db	3Db

Research Parameters

Erythrocyte

Blood is sucked with a pipette containing red stirring grains to a scale of 1. Then Hayem's solution (functions to kill white blood cells) is added to a scale of 101. Mixing the blood in the pipette is done by swinging the hand for 3-5 minutes so that the blood is mixed evenly. After that, the first and second drops of blood solution in the pipette were discarded, then dripped on the Neubauer type haemacytometer and covered with a cover glass (Andayani et al., 2017). Erythrocytes are counted as 5 small squares and the number is calculated according to the formula:

Erythrocytes (cells/mm3) = Number of erythrocyte cells x 104

Leukocyte

The procedure for calculating leukocytes according to Andayani et al., (2017), is to take a sample of fish blood using a thoma pipette, white stirrer grains to a scale of 0.5. Then Turk's solution was added to a scale of 11. The blood was mixed slowly so as not to damage the blood cells by swinging to form a figure eight for 5 minutes. The first drop of blood in the pipette is discarded as much as 2 drops which are assumed to be inhomogeneous. Then the blood was dripped on the haemocytometer and covered with a cover glass. Leukocytes were observed using a microscope with a magnification of 400 times. Counting the number of leukocytes counted as many as 4 small squares, starting from the upper left corner, then to the right, then down and from the right then to the left. The number of leukocytes is calculated by the formula:

Leukocytes (cells/mm3) = Number of leukocyte cells X 50

Hematocrit

Take a sample of tilapia blood using a 1 cc syringe, as much as ± 1 ml. Collection of blood through the caudal vein. The blood sample is then inserted into the microhematocrit tube until it reaches ¾ of the section, then it is plugged at the end using cretoseal. The blood sample was centrifuged at 5,000 rpm for 5 minutes (Andayani et al., 2017).

Hemoglobin

Hemoglobin levels were measured using the Sahli method. A total of 0.1 N-HCl was put into the dilution tube to show a scale of 2. The blood was sucked with an Hb pipette to a scale of 20, then stirred using a stir bar. The diluent tube is inserted into the block comparator to compare the color of the blood solution with the standard solution beside it, if it is not the same, add distilled water drop by drop into the diluent tube until the blood solution is the same as the standard solution. The height of the blood solution on the scale is calculated as the Hb level (g/dL) (Alipin & Sari, 2020).

Data analysis

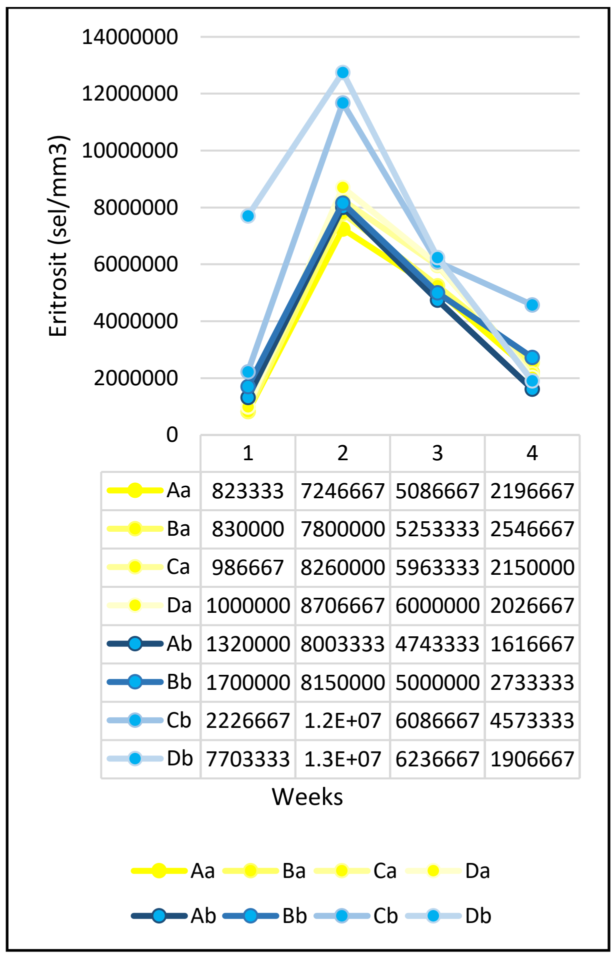
The data obtained from the observations are presented in the form of tables and graphs. The design used was Factorial Completely Randomized Design. Furthermore, the data obtained were analyzed using Microsoft Exel, Factorial Analysis ANOVA at 95% confidence interval.

Results and Discussion

Erythrocyte

The results of the 4-week study had a significant effect on the value of tilapia erythrocytes, which can be seen in Figure 2.

Figure 2. Erythrocyte measurement of red tilapia for 4 weeks, Aa (2 fish/10L with water spinach), Ba (4 fish/10L with water spinach), Ca (6 fish/10L with water spinach), Da (8 fish/10L with water spinach), Ab (2 fish/10L without water spinach), Bb (4 fish/10L without water spinach), Cb (6 fish/10L without water spinach), and Db (8 fish/10L without water spinach).



	1	2	3	4
Aa	823333	7246667	5086667	2196667
Ba	830000	7800000	5253333	2546667
Ca	986667	8260000	5963333	2150000
Da	1000000	8706667	6000000	2026667
Ab	1320000	8003333	4743333	1616667
Bb	1700000	8150000	5000000	2733333
Cb	2226667	1.2E+07	6086667	4573333
Db	7703333	1.3E+07	6236667	1906667

Based on the picture above, it is known that the observation of erythrocytes in each treatment from week 1 to week 4 experienced fluctuations. Erythrocytes in all treatments experienced the highest point in the 2nd week. Erythrocytes decreased in the 3rd and 4th weeks. It was estimated that the fish experienced a peak of stress in general in all treatments, experiencing an increase in erythrocytes along with the amount of stocking density was during the 2nd week. In the 2nd week all treatments showed erythrocyte values exceeding the normal range. According to Dianti et al (2013), the number of erythrocytes in normal tilapia generally ranges from 1.05 – 3.0 x 106 cells/mm3. If the erythrocyte value exceeds the normal range, then the fish is in a state of stress. Total erythrocytes that are too high indicate the fish are in a state of stress so that the fish produce new erythrocytes (Arlanda et al., 2018). According to Fauzan et al., (2017) which states that a high number of erythrocytes indicates that the fish is in a state of stress.

Stress causes fish to need large amounts of energy to maintain balance in their bodies. This energy is produced from the breakdown of glycogen in anaerobic metabolic processes which are regulated by the hormone cortisol (Mommsen et al., 1999). This metabolism causes the need for oxygen to increase and triggers fish to experience hypoxia. Anaerobic metabolism also causes CO2 production to increase. These two things cause fish to increase the number of red blood cells to increase oxygen supply and accelerate the process of CO2 excretion out of the body. This condition causes chromaffin cells to release catecholamine hormones as a primary response to stress into the blood which aims to overcome disturbances in homeostatic and metabolic balance that occur due to stress, one of which is reduced oxygen content in the blood. The increase in catecholamine hormones functions to increase the affinity and capacity of blood to transport oxygen. This increase causes an increase in the number of erythrocytes, hemoglobin levels, and hematocrit (Supriyono et al., 2015).

At 4th week, all treatments experienced a decrease in the number of erythrocytes. This proves that at the 4th week of tilapia reared in media buckets have experienced a decrease in stress levels. In the 4th week, the erythrocyte values in all treatments were normal except for the Cb treatment (6 fish/10L without water spinach). The best treatment was Ab (2 fish/10L without water spinach) which was 1,616,666 cells/mm3. The condition of the treated fish had adapted to the stress and would recover to the initial homeostasis. Fish have a high adaptability to their environment (Sinansari et al., 2021). The treatment without water spinach with the lowest stocking density recovered the number of erythrocytes faster than the treatment with a higher stocking density, Budikdamber with water spinach. It is suspected that the Budikdamber treatment with water spinach did not significantly express the state of stress in its metabolism, namely the number of erythrocytes when compared to the Budikdamber treatment without water spinach.

To determine the effect of differences in erythrocytes between treatments, a test of variance was performed which is presented in Table 2.

Table 2. Erythrocyte Variety Fingerprint Test

Sources of diversity	Db	JK	KT	F Count	F Table
					5%
Treatment	7	30407949739583,30	4343992819940,47	118,32*	2,66
A (Density)	3	13502515364583,30	4500838454861,10	122,59*	3,24

B (System)	1	9472125260416,62	9472125260416,62	258,00*	4,49
AB (DensityXSystem)	3	7433309114583,37	2477769704861,12	67,49*	3,24
Error	16	587425000000,00	36714062500,00		
Total	23	30995374739583,30			

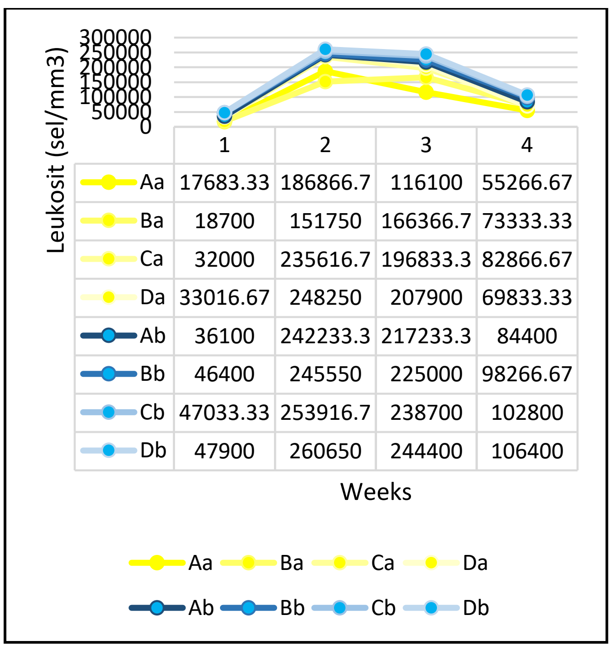
Remarks: (*) Significant

The results of the analysis of variance for treatments A (density), B (system) and AB interactions have F count greater than F table 5%, so it can be concluded that the treatment of factors A, B, and their interactions (AxB) has a real or significant effect.

Leukocytes

The results of the 4th week study had a significant effect on leukocyte values in tilapia, which can be seen in Figure 3.

Figure 3. Leukocyte Measurement of Red Tilapia for 4 Weeks, Aa (2 fish/10L with water spinach), Ba (4 fish/10L with water spinach), Ca (6 fish/10L with water spinach), Da (8 fish/10L with water spinach), Ab (2 fish/10L without water spinach), Bb (4 fish/10L without water spinach), Cb (6 fish/10L without water spinach), and Db (8 fish/10L without water spinach).



Based on Figure 3, it is known that the observation of leukocytes in each treatment from week 1 to week 4 experienced fluctuations. Leukocytes in all treatments experienced the highest point in the 2nd week. Leukocytes decreased in the 3rd and 4th weeks. It was estimated that the fish experienced peak stress in general (all treatments) during the 2nd week. In the 2nd week all treatments showed leukocyte values above the normal range. According to Lagler et al., (1977) the normal range of tilapia leukocytes is 20,000 cells/mm3 -150,000 cells/mm3. Sick fish will produce many leukocytes to phagocytize bacteria and synthesize antibodies (Royan et al., 2014). If the leukocyte value exceeds the normal range, then the fish is in a state of stress. An increase in total white blood cells indicates that fish are experiencing stress (Supriyono et al., 2015).

The stress experienced by tilapia causes pressure on the function of the fish's immune system (Bonga, 1997). Pressure on the function of the immune system makes it easier for pathogens that do not cause disease when normal fish conditions can attack fish during the rearing period in the Budikdamber system. These conditions cause white blood cells to increase which is a response to pathogen attacks that occur (Blaxhall, 1972).

At 4th week, all treatments experienced a decrease in the number of leukocytes. This proves that during the 4th week of tilapia reared in media buckets experienced a decrease in stress levels. In the 4th week, the leukocyte values in all treatments showed a normal state with the best treatment being Aa (stocking density of 2 fish/10L with water spinach) which had a leukocyte value of

55,266.67 cells/mm3. This condition indicated that the treated fish had adapted to stress and would recover to their initial homeostasis. In general, animals have a high adaptability to their environment to maintain their survival, as well as fish (Sinansari et al., 2021). Rearing of Budikdamber with water spinach can make the fish feel safer because they are protected by water spinach which plays a role in phytoremediation, namely reducing, extracting, or eliminating organic and inorganic compounds in the cultivation so that the quality of the rearing water becomes optimal and recovery from stress to initial haemostasis is faster than cultivation without water spinach. (Effendi et al., 2015).

To determine the effect of differences in leukocytes between treatments, a test of variance was performed which is presented in Table 3.

Table 3. Leukocyte Variety Fingerprint Test

Sources of Diversity	Db	JK	KT	F Count	F Table
					5%
Treatment	7	11561121347,66	1651588763,95	79,32*	2,66
A (Density)	3	3965458977,86	1321819659,29	63,48*	3,24
B (System)	1	6882130006,51	6882130006,51	330,52*	4,49
AB (DensityXSistem)	3	713532363,28	237844121,09	11,42*	3,24
Error	16	333157708,33	20822356,77		
Total	23	11894279055,99			

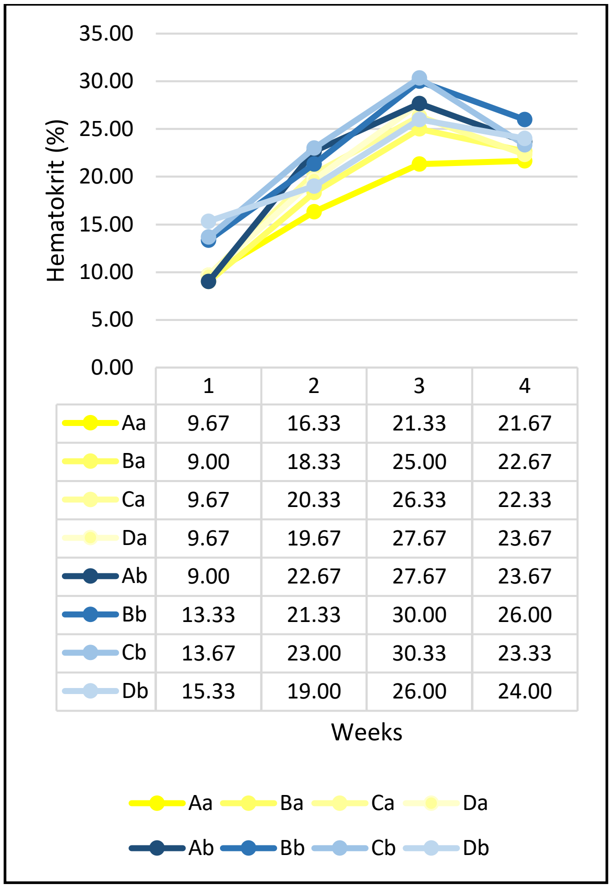
Remarks: (*) Significant

The results of leukocyte analysis of variance in treatment A (density), B (system) and their interactions (AxB) have F count greater than F Table 5%, so it can be concluded that the treatment of factors A, B, and their interactions AxB has a real or significant effect.

Hematocrit

The results of the 4th week study had a significant effect on blood hematocrit in tilapia, which can be seen in Figure 4.

Figure 4. Hematocrit measurement of red tilapia for 4 weeks, Aa (2 fish/10L with water spinach), Ba (4 fish/10L with water spinach), Ca (6 fish/10L with water spinach), Da (8 fish/10L with water spinach), Ab (2 fish/10L without water spinach), Bb (4 fish/10L without water spinach), Cb (6 fish/10L without water spinach), and Db (8 fish/10L without water spinach).



	1	2	3	4
Aa	9.67	16.33	21.33	21.67
Ba	9.00	18.33	25.00	22.67
Ca	9.67	20.33	26.33	22.33
Da	9.67	19.67	27.67	23.67
Ab	9.00	22.67	27.67	23.67
Bb	13.33	21.33	30.00	26.00
Cb	13.67	23.00	30.33	23.33
Db	15.33	19.00	26.00	24.00

Based on Figure 4, it is known that the observation of the hematocrit in each treatment from week 1 to week 4 fluctuated. The hematocrit in all treatments experienced its highest point in the 3rd week. The hematocrit decreased in all treatments except for treatment Aa which decreased in the 4th week. It was estimated that the fish experienced peak stress in general (all treatments) during the 3rd week. In the 3rd week, all treatments except Aa (stocking density of 2 fish/10L with water spinach) showed a hematocrit value above the normal range. According to Royan et al (2014), the value of hematocrit levels in tilapia ranges from 21.00% - 22.67%. If the hematocrit value exceeds the normal range, then the fish is in a state of stress. This metabolism causes the need for oxygen to increase and triggers fish to experience hypoxia (Delince et al., 1987).

Anaerobic metabolism also causes CO2 production to increase. These two things cause fish to increase the number of red blood cells to increase oxygen supply and speed up the process of CO2 excretion out of the body. This condition causes chromaffin cells to release catecholamine hormones as a primary response to stress into the blood which aims to overcome disturbances in homeostatic and metabolic balance that occur due to stress, one of which is reduced oxygen content in the blood. The increase in catecholamine hormones functions to increase the affinity and capacity of blood to transport oxygen. This increase causes an increase in the number of red blood cells, hemoglobin levels, and the hematocrit value of fish.

At 4th week, all treatments experienced a decrease in the number of hematocrit. In the 4th week, the hematocrit values in all treatments with water spinach except Da (stocking density of 8 fish/10L) were normal. The best treatment was Aa (stocking density of 2 fish/10L with water spinach) which had a hematocrit value of 21.67%. This condition indicated that the treated fish had adapted to stress and would recover to their initial homeostasis. In general, fish have a high adaptability to their environment to maintain their survival (Sinansaril et al., 2021). In the treatment with water spinach, there was a significant decrease close to normal values when compared to the treatment without water spinach. This indicates that the recovery to normal conditions from the treatment with water spinach was faster than the treatment without water spinach. Rearing of Budikdamber with water spinach can cause the fish to feel safer because they are sheltered by water spinach which plays a role in phytoremediation, namely reducing, extracting, or eliminating organic and inorganic compounds in the cultivation so that water quality becomes more optimal and recovery from stress to initial haemostasis is faster than Budikdamber without it. water spinach (Effendi et al., 2015). To determine the effect of differences in hematocrit between treatments, a test of variance was performed which is presented in Table 4.

Table 4. Hematocrit Multiple Fingerprint Test

Sources of diversity	Db	JK	KT	F Count	F Table
					5%
Treatment	7	70,28	10,04	1.89^{ns}	2,66
A (Density)	3	16,97	5,66	1.07^{ns}	3,24
B (System)	1	45,38	45,38	8,56*	4,49
AB (DensityXSystem)	3	7,94	2,65	0.50^{ns}	3,24
Error	16	84,83	5,30		
Total	23	155,11			

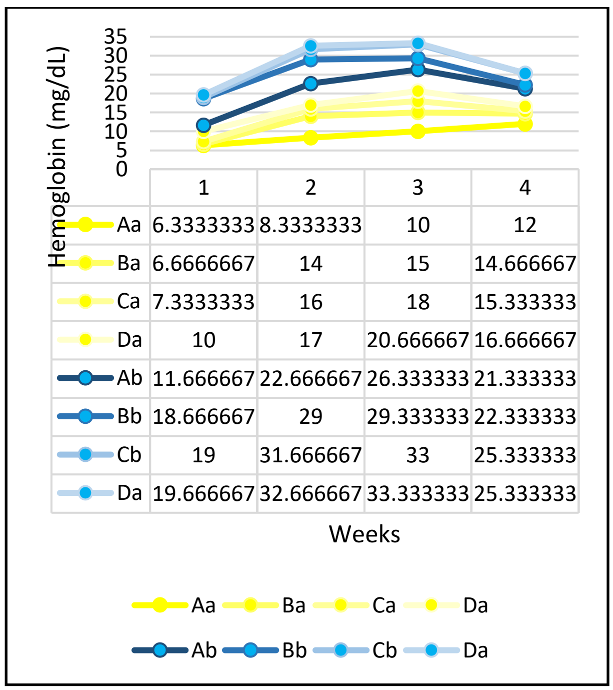
Description: (ns) Non-Significant; (*) Significant

The results of the analysis of variance for treatment A (density) and the interaction of AxB had a calculated F value that was less than Ftable 5%, so it could be concluded that treatment A and its interaction AxB had no significant or non-significant effect.

Hemoglobin

The results of the 4-week study with the treatment of differences in stocking densities in the Budikdamber system with and without water spinach gave a significant effect on blood hemoglobin in tilapia, can be seen in Figure 5.

Figure 5. Hemoglobin Measurement of Red Tilapia for 4 weeks, Aa (2 fish/10L with water spinach), Ba (4 fish/10L with water spinach), Ca (6 fish/10L with water spinach), Da (8 fish/10L with water spinach), Ab (2 fish/10L without water spinach), Bb (4 fish/10L without water spinach), Cb (6 fish/10L without water spinach), and Db (8 fish/10L without water spinach).



Based on Figure 5, it is known that the hemoglobin observed in each treatment from week 1 to week 4 fluctuated. Hemoglobin in all treatments experienced its highest point in the 3rd week. Hemoglobin in all treatments decreased except for treatment Aa in the 4th week. It was estimated that the fish experienced peak stress in general (all treatments) during the 3rd week. In the 3rd week, all treatments except the Aa treatment (stocking density of 2 fish/10L) showed hemoglobin values above the normal range. According to Wedemeyer and Yasutake (1997), normal hemoglobin levels in tilapia range from 10 to 11.1 g/dL. If the hemoglobin value exceeds the normal range, then the fish is in a state of stress. Hemoglobin levels increased above the normal range because fish experienced increased stress conditions (Royan et al., 2021). According to Lagler et al., (1977) the concentration of hemoglobin in the blood is strongly correlated with the number of erythrocytes. The lower the number of erythrocytes, the lower the concentration of hemoglobin in the blood.

At 4th week 4, all treatments experienced a decrease in hemoglobin values except for treatment Aa (stocking density of 2 fish/10L). In the 4th week all treatments showed hemoglobin values within the normal range. This proves that during the 4th week the tilapia reared in the media bucket had already experienced adaptation to the media environment, thereby reducing the stress condition of the fish. The best treatment was Aa (stocking density of 2 fish/10L with water spinach) which had a hemoglobin value of 12 g/dL. The treated fish had adapted to the stress and would recover to their initial homeostasis. In general, animals have high adaptability to their environment to maintain their survival, as well as fish (Sinansari et al., 2021). In the treatment with water spinach, there was a significant decrease close to normal values when compared to the treatment without water spinach. This indicates that the recovery to normal conditions from the treatment with water spinach was faster than the treatment without water spinach. Rearing of Budikdamber with water spinach can cause the fish to feel safer because they are sheltered by water spinach which plays a role in phytoremediation, namely reducing, extracting, or eliminating organic and inorganic compounds in the cultivation so that water quality becomes more optimal and recovery from stress to initial haemostasis is faster than Budikdamber without it. water spinach (Effendi et al., 2015).

To determine the effect of differences in hemoglobin between treatments, a test of variance was carried out which is presented in Table 5.

Table 5. Hemoglobin Multiple Test Test

Sources of diversity	Db	JK	KT	F Count	F Table
					5%
Treatment	7	1051,88	150,27	31,55*	2,66
A (Density)	3	173,19	57,73	12,12*	3,24
B (System)	1	876,04	876,04	183,93*	4,49
AB (DensityXSistem)	3	2,65	0,88	0.19^{ns}	3,24
Error	16	76,21	4,76		
Total	23	1128,08			

Description: (ns) Non-Significant; (*) Significant

The results of the analysis of variance for the AxB interaction treatment had a calculated F value that was less than 5% F table, so it could be concluded that treatment A and its interaction with AxB had no significant or non-significant effect.

Conclusion

Based on the research that has been carried out, it can be concluded that the treatment of differences in stocking density of red tilapia in the Budikdamber system (with water spinach and without water spinach) has a significant effect on changes in fish physiology including hematological parameters (erythrocytes, leukocytes, hematocrit and hemoglobin) for red tilapia and water spinach which indicates that the fish is experiencing stress because it has a hematologic value that is inappropriate and above the normal range. The best and optimal treatment was the Aa treatment (2 fish/10L with water spinach).

Reference

Affandi, R. dan U. M. Tang. (2002). Fisiologi Hewan Air. Unrl Press.

Alipin, K., & Sari, T. A. (2020). Indikator Kesehatan Ikan Kerapu Cantik (Epinephelus sp.) Yang Terdapat Pada Budidaya Keramba Pantai Timur Pangandaran. Metamorfosa: Journal of Biological Sciences, 7(2), 141. https://doi.org/10.24843/metamorfosa.2020.v07.i02.p18

Andayani, S., Suprastyani, H., Gumala, G. D. A., Oktafa, U., Fatikah, N. M., Wahyudi, M., & Pratama, R. (2017). Pengaruh pemberian bakteri Lactobacillus plantarum terhadap histopatologi dan hematologi ikan patin jambal (Pangasius djambal) yang diinfeksi bakteri Edwarsiella tarda. Journal of Fisheries and Marine Science, 1(4), 31-38.

Arianto, D., Harris, H., Anggraini Yusanti, I., & Arumwati, A. (2019). Padat Penebaran Berbeda Terhadap Kelangsungan Hidup, FCR dan Pertumbuhan Ikan Bawal Air Tawar (Colossoma Macropomum) Pada Pemeliharaan Di Waring. Jurnal Ilmu-Ilmu Perikanan Dan Budidaya Perairan, 14(2). https://doi.org/10.31851/jipbp.v14i2.3486

Arlanda, R. Tarsim, dan D.S.C. Utomo. (2018). Pengaruh Pemberian Ekstrak Tembakau (Nicotina tobacum) Sebagai Bahan Anestesi Terhadap Kondisi Hematologi Ikan Nila (Oreochormis niloticus). Jurnal Sains Teknologi Akuakultur. 2(2): 32-40.

Barton, B. A. (2002). Stres in fishes: A diversity of responses with particular reference to changes in circulating corticosteroids. Integrative and Comparative Biology, 42(3), 517–525. https://doi.org/10.1093/icb/42.3.517

Bonga SW. (1997). The stres response in fish. Physiological Review,77(3): 591-625.

Delince GA, Campbell D, Janssen JAL, Kutty MN. 1987. Seed production. Lectures presented at ARAC (African Regional Aquaculture Centre) for the senior aquaculturists course. Field document. Report No: FAO-FI--RAF/82/009. Food and Agriculture Organization of the United Nations, Rome. 118 p.

Dianti, L., S. B. Prayitno dan R. W. Ariyanti. (2013). Ketahanan Nonspesifik Ikan Mas (Cyprinus carpio) yang Direndam Ekstrak Dun Jeruju Terhadap Infeksi Bakteri Aeromonas hydrophila. Journal of Aquaculture Management and Technology. 2(4): 63-71.

Effendi H., Utomo B.A., Darmawangsa G.M., & Karo-Karo R.E. (2015). Fitoremediasi Limbah Budidaya Ikan Lele (Clarias sp.) dengan Kangkung (Ipomoea aquatica) dan Pakcoy (Brassica rapa chinensis) dalam Sistem Resirkulasi. Ecolab. 9(2):80-92

Fauzan, M., Rosmaidar., Sugito., Zuhrawati., Muttaqien dan Azhar. (2017). Pengaruh Tingkat Paparan Timbal (Pb) Terhadap Profil Darah Ikan Nila (Oreocromis niloticus). Jurnal Ilmiah Mahasiswa Veteriner. 1(4): 702-708.

Haqqalwy, E.J., Winaruddin, D.A., dan Hamdani, B. (2013). Pengaruh Kepadatan Polpulasi Terhadap Gambaran Patologi Anatomi Dan Histologi Insang Ikan Nila (Oreochormis niloticus). Jurnal Medika Veterinaria. 7(1); 25-26.

Ismail, K. (1994). Kiat Mengatasi Stres pada Ikan. Mediatama.

Lagler, K.F., Bardach, J.E., R.R., Miller, dan Passino, D.R.M. 1977. Ichthyology. John Willey and Sons. Inc. New York-London.

Mommsen TP, Vijayan MM, Moon TW. (1999). Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. Reviews in Fish Biology and Fisheries, 9(3): 211-268

Ni, M., Wen, H., Li, J., Chi, M., Bu, Y., Ren, Y., Zhang, M., Song, Z., & Houmeng. (2014). The physiological performance and immune responses of juvenile Amur sturgeon (Acipenser schrenckii) to stocking density and hypoxia stres. Fish & Shellfish Immunology, 36(2), 325–335.

Nugroho, E, dan Sutrisno. 2008. Budidaya Ikan dan Sayuran dengan Sistem Akuaponik. Penebar Swadaya. Jakarta.

Prihadi, T. H., Saputra, A., Huwoyon, G. H., & Pantjara, B. (2017). Gambaran Darah Benih Ikan Betutu Oxyeleotris marmorata. Jurnal Penyuluhan Perikanan Dan Kelautan, 12(1), 341–350.

Royan, F., S. Rejeki dan A.H.C. Haditomo. (2014). Pengaruh Salinitas Yang Berbeda Terhadap Profil Darah Ikan Nila (Oreocromis niloticus). Journal Aquaculture Management and Technology. 3(2): 109-117.

Sarah, S., & Pramulya, R. (2021). Partisipasi Masyarakat terhadap Program Budidaya Ikan dalam Ember (Budikdamber) Solusi Ketersediaan Bahan Pangan. Jurnal Pendidikan Tambusai, 5(3), 11245–11258.

Sinansari, S., Prakoso, V. A., Hayuningtyas, E. P., Priadi, B., Sundari, S., & Kusrini, E. (2021). Pengaruh Padat Tebar terhadap Konsumsi Oksigen dan Respons Stres Ikan Cupang Alam (Betta imbellis). OLDI (Oseanologi dan Limnologi di Indonesia), 6(1), 11-24.

Sucipto dan Prihartono (2007), Pembesaran Nila Hitam Bangkok di Karamba Jaring Apung, Kolam Air Deras, Kolam Air Tenang dan Karamba. Penerbit Penebar Swadaya, Jakarta.

Supriyono, E., Nirmala, K., & Harris, E. (2015). Pengaruh Kepadatan Ikan Selama Pengangkutan terhadap Gambaran Darah, PH Darah, dan Kelangsungan Hidup Benih Ikan Gabus Channa Striata (Bloch, 1793). Jurnal Iktiologi Indonesia, 15(2), 165-177.

Wedemeyer, G.A and Yasutke. 1977. Clinical Methods for The Assessment on The Effect of Enviromental Stres on Fish Health. Technical Paper of The US Departement of The Interior Fish and the Wildlife Service, 89 : 1-17.

Yuni, K. P., H. Hasam dan E. Prasetio. (2019). Studi Hematologi ikan semah (Tordouronensis), jelawat (Leptobarbus hoeveni), tengadak (Barbonymus schwanenfeldi). biawan (Helostoma temmincki), dan botia (Chromobotia macracanthus). Jurnal Ruaya. 7 (1) : 65-69.