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RESPONSE OF STOCKING DENSITY DIFFERENCES TO KIDNEY HISTOLOGY ANALYSIS OF RED TILAPIA (*Oreochromis* sp.) IN THE BUDIKDAMBER SYSTEM

Respons Perbedaan Kepadatan Tebar Terhadap Analisis Histologi Ginjal Ikan Nila Merah (*Oreochromis* sp.) Dalam Sistem Budikdamber

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ABSTRACT

Optimizing high-density fish farming along with increased feeding will lead to the accumulation of organic matter. This accumulation of organic matter (such as leftover feed and feces) will deteriorate maintenance conditions, ultimately affecting the survival and growth of the fish. The physiological condition of fish due to unsuitable environment will cause changes in histological condition of tissues and organs that play a role in the metabolism of the fish. The aim of this study was to examine how variations in stocking density affect the histological analysis of the kidneys of red tilapia (*Oreochromis sp.*) raised in the budikdamber system. The study was conducted for 28 days in the reproduction and hydrobiology laboratory of Brawijaya University. This study employed a completely randomized factorial design with density treatments (A (2 fish/10L), B (4 fish/10L), C (6 fish/10L), D (8 fish/10L) and system (budikdamber with kale (a) and without kale (b)). Some kidney organ damage is congestion, edema and necrosis. The most effective treatmenttreatment is Ab (2 fish/10L without kale) which has the least damage value.

Keywords: Budikdamber, High Stocking Density, Kidney Histology

ABSTRAK

Optimalisasi budidaya ikan dengan kepadatan tinggi bersamaan dengan peningkatan pemberian pakan akan menyebabkan penumpukan bahan organik. Penumpukan bahan organik ini seperti sisa pakan dan feses akan memperburuk kondisi pemeliharaan, yang pada akhirnya memengaruhi kelangsungan hidup dan pertumbuhan ikan. Kondisi fisiologis ikan akibat lingkungan yang tidak sesuai akan menyebabkan terjadinya perubahan kondisi histologis pada jaringan dan organ yang berperan dalam metabolisme ikan tersebut. Tujuan penelitian ini adalah menganalisis respon perbedaan padat tebar terhadap analisis histologis ginjal ikan nila merah (*Oreochromis sp*) yang dipelihara menggunakan sistem budikdamber. Penelitian dilakukan selama 28 hari di laboratorium reproduksi dan hidrobiologi Universitas Brawijaya. Pada penelitian ini menggunakan rancangan acak lengkap faktorial dengan perlakuan kepadatan (A (2 ekor/10L), B (4 ekor/10L), C (6 ekor/10L), D (8 ekor/10L) dan sistem (budikdamber dengan kangkung (a) dan tanpa kangkung (b)). Beberapa kerusakan organ ginjal yaitu kongesti, edema dan nekrosis. Perlakuan terbaik adalah Ab (2 ekor/10L tanpa kangkung) yaitu dengan nilai kerusakan paling sedikit.

Kata kunci: Budikdamber, Histologi Ginjal, Padat Tebar Tinggi

INTRODUCTION

The development of plant, fruit, and fish cultivation is increasingly facing various challenges, including limited land and decreasing quality and quantity of water resources (Susetya & Harahap, 2018). Global problems related to decreasing quality and quantity of water for human needs, limited food sources, and increasing world population growth (Nursandi, 2018). Optimizing fish cultivation with high stocking density and large amount of feed can result in accumulation of organic matter in aquaculture practices. Accumulation of organic matter such as leftover feed and feces will reduce water quality in maintenance, which can ultimately affect the survival and growth of fish including the histology of the fish (Djauhari *et al.,* 2019). Increasing high fish stocking density can disrupt the physiological processes and behavior of fish in utilizing their movement space. As a result, the health condition and physiological function of fish decline, which in turn has an impact on reduced efficiency of feed utilization and stunted growth (Anwar *et al.,* 2022). Stocking density treatment in fish will also affect the degree of survival and growth of fish which leads to stress (Adineh *et al.,* 2019; Nadiro *et al.,* 2023; Ofori-Mensah *et al.*, 2018). In addition to stress, the physiological condition of fish due to an unsuitable environment will cause changes in the histological condition of the tissues and organs that play a role in the metabolism of the fish (Prihadi *et al.*, 2017).

Diagnosis of disease in fish must consider clinical signs, including external and internal characteristics and pathological changes. Histopathological examination is carried out to observe tissue changes that arise due to infection that causes abnormalities in the tissue (Asrido *et al.,* 2024). Histological analysis is a method used to assess various abnormalities and changes in fish organ tissue (Esmaeilbeigi *et al.,* 2021). Histological analysis is considered as one of the useful methods to determine damage to tissues and organs due to environmental changes. Histological analysis methods can also help in identifying and determining lesions that appear in certain fish organs that are exposed to or experience environmental changes (Iftikhar *et al.,* 2022).

The kidney is the main organ in fish that can be affected by environmental changes due to differences in stocking density in cultivation which will then affect fish habits, fish growth, swimming ability, and metabolism in fish. When fish experience stress, they will release the hormone cortisol. The hormone cortisol is released from the interrenal tissue located in the kidneys (anterior part) in response to several pituitary hormones, especially adrenocorticotropic hormone (ACTH) (Lestari & Syukriah, 2020). The kidney organ plays a role in the metabolic process in the fish's body. In addition, the kidneys also function as organs that produce blood in fish (Hasibuan *et al.,* 2021). If the stocking density exceeds the optimum capacity, the fish's metabolism will automatically be affected, including the fish's blood flow.

Based on this explanation, tilapia cultivation in buckets must be carried out effectively and efficiently in order to increase production capacity by applying the most optimal stocking density. Thus, further research is needed regarding the best tilapia stocking density in the budikdamber system, with a focus on histological analysis of the kidneys of red tilapia.

RESEARCH METHODS

Research Location

This research was conducted for 28 days (4 weeks) in January 2023. The research location was at the Fish Reproduction and Hydrobiology Laboratory, Faculty of Fisheries and Marine Sciences, Brawijaya University, Malang as shown in Figure 1.

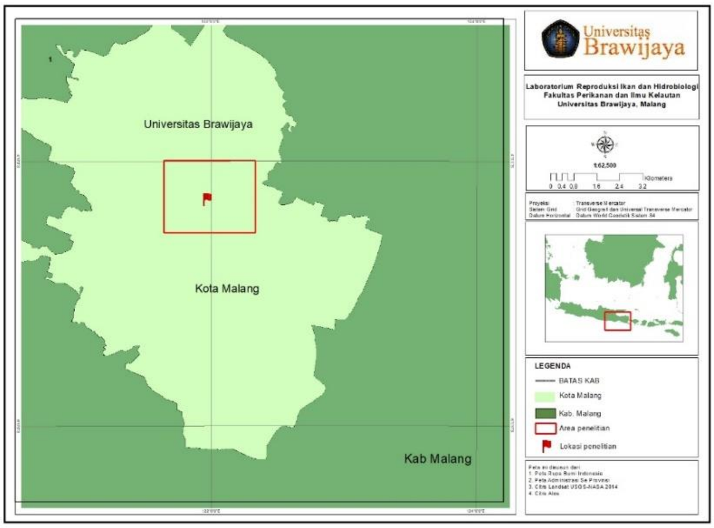

Figure 1. Fish Reproduction Laboratory, Faculty of Fisheries and Marine Sciences, Brawijaya University, Malang

Research Methods

The stocking density of fish in the budikdamber with a volume of 80 liters is 60-100 catfish (SOP Budikdamber, 2020). For the type of tilapia that is maintained in the budikdamber system, it is currently still in the development stage. According to Fadly and Henggu (2021), the stocking density of tilapia in the budikdamber system is 1 fish/2 liters with a survival rate of 100%. The experimental design in this experiment used a completely randomized factorial design with density treatments (A (2 fish/10L), B (4 fish/10L), C (6 fish/10L), D (8 fish/10L) and system (budikdamber with kale (a) and without kale (b)). The experimental layout used in this study can be seen in Table 1.

Table 1. Research Design

Density	System	repetition		
		1	2	3
A (2 fish/10L)	With Water Spinach (a)	1Aa	2Aa	3Aa
	Without Water Spinach (b)	1Ab	2Ab	3Ab
B (4 fish/10L)	With Water Spinach (a)	1Ba	2Ba	3Ba
	Without Water Spinach (b)	1Bb	2Bb	3Bb
C (6 fish/10L)	With Water Spinach (a)	1Ca	2Ca	3Ca
	Without Water Spinach (b)	1Cb	2Cb	3Cb
D (8 fish/10L)	With Water Spinach (a)	1Da	2Da	3Da
	Without Water Spinach (b)	1Db	2Db	3Db

Tools and Materials

The tools used in this study were water spinach seedling tools (rockwool, tweezers, trays), 16 liter buckets, used plastic cups, wire, paranet, DO meter, pH meter, thermometer, embedding tissue, microtome, oven, waterbath, hot plate, electron microscope, refrigerator, beaker, petri dish, slide tweezers, cover glass. The materials used in this study were water spinach seeds, fresh water, 6 cm red tilapia, PF1000 feed, Davidson solution, alcohol (70%, 80%, 85%, 100%), xylene, paraffin, hematoxylin, eosin, entenen, tissue, label paper, and red tilapia kidney.

Histology Examination Process

Kidney tissue sampling was carried out on the 28th day. The tissue taken was cleaned with distilled water, then inserted into a microtube containing Davidson's solution. Furthermore, histopathological preparations were made. The histopathological examination process includes fixation, dehydration, clearing, impregnation, embedding, staining, and mouthing. Histological observations were carried out with the aim of seeing the picture of kidney tissue in tilapia treated with different stocking densities in the budikdamber system, then scoring was carried out for damage. According to (Izzah *et al.*, 2019), Scoring is the process of assessing each parameter to determine tissue conditions by calculating the level of damage in percentage form. Reading starts from the left side (according to the position of the tail of the preparation) towards the head, then down and shifting back towards the tail in a zig-zag pattern. Each field of view was examined to determine the level of tissue damage based on the criteria of hemorrhage, congestion, and necrosis, then the percentage was given a score between 1 and 4. The scoring values according to (Maftuch *et al.*, 2015), against the percentage of organ damage are presented in Table 2.

Table 2. Histology Scoring

Scoring Value	Percentage of Damage (%)	Description
0	0	No damage
1	1-25	Slight damage
2	26-50	Moderate damage
3	51-75	A lot of damage
4	76-100	Very much damage

RESULT

Histology of Red Tilapia Kidney

The histology of the kidneys of red tilapia in the control can be seen in Figure 2.

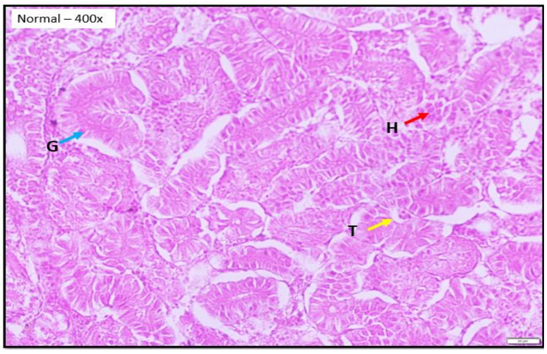

Figure 2. Normal (healthy) Kidney of Red Tilapia Fish with 400x Microscope Magnification. Caption: Blue Arrow = Glomerulus, Yellow Arrow = Tubule, Red Arrow = Haemopotik

The histological results of the kidney organs of red tilapia fish after treatment with different stocking densities in the budikdamber media for 28 days (4 weeks) can be seen in Figure 3.

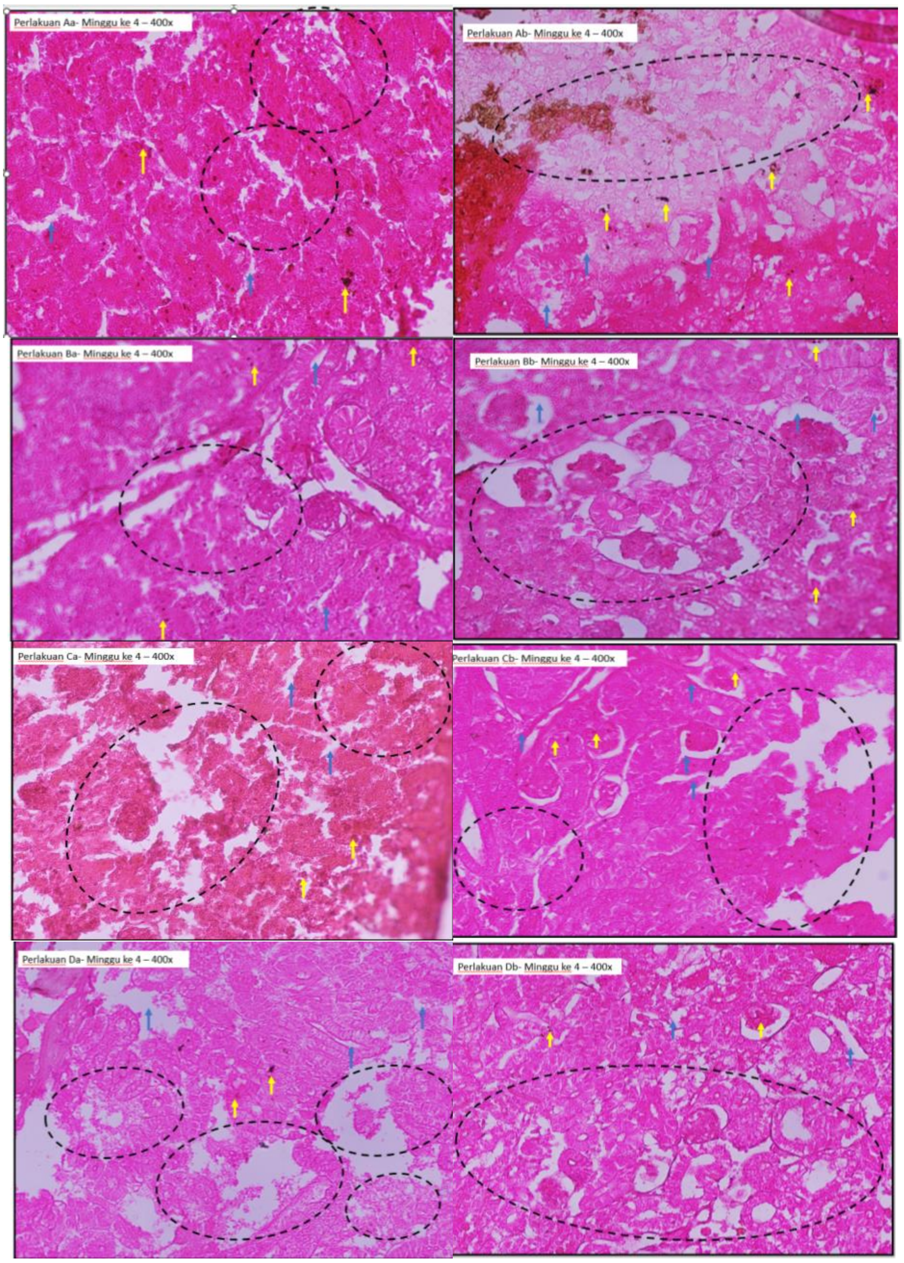

Figure 3. Histology of Red Tilapia Kidney After 28 Days of Treatment, 400x Microscope Magnification. Caption: Blue Arrow = Edema, Yellow Arrow = Congestion, Dotted Circle = Necrosis

Analysis of kidney organ damage in red tilapia due to different stocking density treatments in the budikdamber system and different systems (with and without kale) on the 28th day (4th week) is as follows:

✓ Congestion

Different stocking density treatments in the budikdamber system with and without water spinach resulted in congestion damage scoring which is presented in Table 3.

Table 3. Scoring of Histological Congestion Damage of Red Tilapia Kidney Organs

Congestion	Average	Description
Aa	2,1	Moderate Damage
Ba	2,2	Moderate Damage
Ca	2,6	Moderate Damage
Da	2,0	Moderate Damage
Ab	1,5	Moderate Damage
Bb	2,1	Moderate Damage
Cb	2,5	Moderate Damage
Db	2,0	Moderate Damage

✓ Edema

Different stocking density treatments in the budikdamber system with and without kale on edema damage scoring are presented in Table 4.

Table 4. Scoring of Histological Edema Damage to the Kidney Organ of Red Tilapia Fish

Edema	Average	Description
Aa	0,8	Minor Damage
Ba	0,3	No Damage
Ca	0,0	No Damage
Da	1,2	Minor Damage
Ab	0,4	No Damage
Bb	1,4	Minor Damage
Cb	1,4	Minor Damage
Db	2,2	Minor Damage

✓ Necrosis

Different stocking density treatments in the budikdamber system with and without water spinach resulted in necrotic damage to the kidney organs of red tilapia fish, as presented in Table 5.

Table 5. Scoring of Histological Necrosis Damage to the Kidney Organ of Red Tilapia Fish

Necrosis	Average	Description
Aa	1,5	Moderate Damage
Ba	2,5	Moderate Damage
Ca	1,9	Moderate Damage
Da	3,0	Heavy Damage
Ab	1,7	Moderate Damage
Bb	2,3	Moderate Damage
Cb	2,3	Minor Damage
Db	3,2	Heavy Damage

DISCUSSION

Figure 3 above shows that the condition of the red tilapia kidney is healthy and normal and does not experience damage. The structure of the red tilapia kidney is still complete, namely there are glomerulus, tubules, and hemopotik. The kidney organ has a function that is in accordance with the series of metabolism in the fish's body. The function of the glomerulus is to absorb protein from the blood, while the tubules are tasked with filtering minerals and water from the blood.

Based on table 3, it shows that the most severe congestion damage is in the Ca treatment (6 fish/10L with kale) with congestion damage of 2.6 (moderate damage) and the lowest damage and the best treatment is Ab (2 fish/10L without kale) namely with a congestion damage value of 1.5 (moderate damage). Congestion is the widening of blood vessels that are filled with blood beyond normal capacity. If this congestion occurs excessively, it can cause hemorrhage. Histological changes characterized by congestion show an increase in blood volume in the vessels, where the blood capillaries look dilated and filled with erythrocytes (Wagiman *et al.,* 2014). This is in accordance with the condition of tilapia in the budikdamber system with or without water spinach which experiences stress with hematology values that exceed normal. Congestion in the kidneys of red tilapia to the treatment of differences in stocking density in the budikdamber system with or without water spinach is caused by poor water conditions and unstable fish metabolism conditions due to stress from stocking density.

In table 4 above, the most severe edema in the Db treatment (8 fish/10L without kale) with edema 2.2 and the lightest damage and also the best treatment is Ca (6 fish/10L with kale) with an edema value of 0. According to (Maftuch *et al.*, 2018), Edema is cell swelling that occurs due to obstruction of blood flow. Edema in kidney tissue can cause damage or shrinkage of tissue parts, which ultimately leads to necrosis. This is in accordance with the condition of red tilapia in the budikdamber system which experiences density stress and is associated with hematological conditions that exceed normal limits (stress).

Based on the table above, it shows that the most severe necrosis damage is in the Db treatment (8 fish/10L without kale) with necrosis damage 2.1 and the lightest damage and also the best treatment is Ab (2 fish/10L without kale) with a necrosis damage value of 1.0. Necrosis refers to a condition in which tissue activity decreases, characterized by the gradual loss of some cells, leading to tissue death. Necrosis is also known as local death of tissue in the body of a living organism. This condition is important to note because damage occurs while the animal is still alive, so it can be used as analysis material to identify the cause of death of the animal (Oktafitria & Maulidina, 2018). According to (Wahyuni & Manda, 2017), necrosis is histologically characterized by the appearance of unclear cell boundaries and cell nuclei that appear faint or even missing. Cell death (necrosis) in the kidneys can affect their performance in the body's metabolic processes. Necrosis in the kidneys can cause hypoxia, which has an impact on metabolic disorders due to reduced oxygen supply to the tissues. In addition, impaired kidney function can also inhibit the physiological processes of fish, especially in blood cleansing and erythropoiesis (Hapsari, 2022). The structure of the kidney organ also shows abnormalities in the form of necrosis. This indicates that the fish are stressed which causes the kidneys to experience damage and cell death, as well as the function of these organs in the metabolism of the fish's body is reduced. The necrosis is caused by stress on the fish due to less than optimal stocking density.

CONCLUSION

Based on the results of the research that has been done, it can be concluded that the difference in stocking density of red tilapia in the budikdamber system (with and without water spinach) has a significant effect on histological changes in the kidney organs of red tilapia. Several types of damage identified include congestion, edema, and necrosis. The best treatment was found in the Ab parameter, namely a stocking density of 2 fish per 10 liters without the use of water spinach.

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