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CARCASS WEIGHT AND SKELETAL MUSCLE MICROSCOPIC STRUCTURE OF RED NILE TILAPIA (*Oreochromis niloticus*) IN THE DIFFERENT AERATION AND FILTRATION

BOBOT KARKAS DAN STRUKTUR MIKROSKOPIS OTOT RANGKA IKAN NILA MERAH (*Oreochromis niloticus*) PADA AERASI DAN FILTRASI BERBEDA

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Abstract

Red tilapia (*Oreochromis niloticus*) is a freshwater fish that widely liked by Indonesian people. Keeping fish with good water quality will increase productivity. The aim of this research was to determine the effect of adding aeration and using filters on carcass weight, muscle fiber diameter and number of muscle fibers in red tilapia. This research used 24 red tilapia fish with an initial body weight of around 7 g. Divided into 4 groups, namely the maintenance group using one aerator without a filter (ANF), the maintenance group using two aerators without a filter (AANF), the maintenance group using one aerator and using a filter (AF) and the maintenance group using two aerators and using a filter (AAF). The results showed that rearing red tilapia fish in the group rearing two aerators and using a filter had a significant effect ($P < 0.05$) on carcass weight, muscle fiber diameter, and number of muscle fibers. Observation of the muscle histology structure showed that there was no damage to the muscle histology structure. The conclusion of this research indicate that additional aerator equipped with filters will supports the growth of red tilapia fish.

Keywords: Aerator; Carcass weight; Filter; Muscle diameter; Number of muscle fibers; Red Nile Tilapia

Abstrak

Ikan nila merah (Oreochromis niloticus) merupakan ikan yang banyak disukai masyarakat Indonesia. Pemeliharaan ikan dengan kualitas air yang baik akan memberikan peningkatan produktivitas. Tujuan penelitian ini adalah mengetahui pengaruh penambahan aerasi dan penggunaan filter terhadap bobot karkas, diameter serabut otot serta jumlah serabut otot pada ikan nila merah. Penelitian ini menggunakan 24 ekor ikan nila merah dengan bobot badan awal berkisar 7 g. Dibagi menjadi 4 kelompok, yaitu kelompok pemeliharaan menggunakan satu aerator tanpa filter (ANF), kelompok pemeliharaan menggunakan dua aerator tanpa filter (AANF), kelompok pemeliharaan menggunakan satu aerator dan menggunakan filter (AF) serta kelompok pemeliharaan menggunakan dua aerator dan menggunakan filter (AAF). Hasil menunjukkan pemeliharaan ikan nila merah pada kelompok pemeliharaan dua aerator dan menggunakan filter berpengaruh nyata ($P < 0,05$) terhadap bobot karkas, diameter serabut otot, dan jumlah serabut otot. Pada pengamatan struktur histologi otot menunjukkan tidak adanya kerusakan struktur histologi otot. Kesimpulan penelitian ini penambahan aerator dilengkapi filter mendukung pertumbuhan ikan nila merah.

***Kata kunci**: Aerator; Bobot karkas; Diameter otot; Filter; Ikan nila; Jumlah serabut otot*

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INTRODUCTION

Red tilapia (*Oreochromis niloticus*) is a popular fish that has high economic value, so it is widely bred by Indonesian people. Fish production can achieve optimal results if the physical and chemical environmental factors in the form of water temperature, pH, and dissolved oxygen (DO) meet the requirements (Bajaj, 2017). Good water quality with sufficient aeration will increase fish growth in treatments with higher levels of stress, namely in media with high density (Lima et al., 2018). Mechanical aeration will improve water quality and reduce residual feed deposits at the bottom of the pond (Tanveer et al., 2018). Red tilapia cultivation needs to pay attention to water quality on a regular basis, namely by checking temperature, pH, and replacing dirty water with clean water regularly. Filtration can be done as an effort to renew dirty water in the aquarium. The basis for using filters in aquariums is to reduce the high nitrogen compounds such as ammonia, nitrites, nitrates which can harm the membranes and blood cells contained in the fish's body. It is necessary to pay attention to the use of filters to minimize the danger caused by high levels of these compounds (Palao et al., 2020). Exposure to high concentrations of ammonia will cause changes in fish metabolic processes, besides that fish will be more susceptible to disease. Ammonia with high concentrations will accumulate in fish body tissues and cause toxic effects (Franklin & Edward, 2019). Ammonia concentration of 0.18 mg/L can cause inhibition of fish growth. The growth pattern will affect body mass (the accumulation of energy and nutrient reserves) for the development of fish body parts related to the calculation of carcass weight (Lingam et al., 2019).

Carcass quality in fish is calculated to determine the amount of meat that can be consumed. Carcass quality characteristics can be determined by calculating the overall body weight of the fish, carcass weight, non-carcass weight, and meat composition to determine the percentage of fat and protein (Hasan et al., 2016). The three main components of muscle, namely muscle fiber, intramuscular connective tissue, and intramuscular fat are interrelated to determine meat quality (Listrat et al., 2016). The type of muscle that dominates the body of the fish is white muscle. About 90% of the muscle tissue contained in the body of the fish is represented by white muscle. The addition of fish body weight can occur due to changes in muscle growth. The growth of muscle tissue involves a combination of enlargement of muscle fibers (hypertrophy) and an increase in the number of muscle cells (hyperplasia), both of which will affect the body weight of the fish at the end of rearing (Robisalmi et al., 2021). Research that explains in detail the effect of using the level of aeration and filtration on the increase in diameter and the number of muscle fibers so that it affects the weight of fish (carcass) is still very limited, so it is important to conduct research with the aim of analyzing the level of aeration, use of filters and a combination of both, on carcass weight, and microscopic structure of red tilapia skeletal muscle.

MATERIALS AND METHODS

The research was conducted for four months. Preparation of skeletal muscle histology preparations was carried out at the Semarang Animal Health Laboratory and histomorphometric observations were carried out at the Basic Biology Laboratory, Faculty of Science and Mathematics, Diponegoro University.

The tools used during this research were Lion Star brand box container type CB 40 with a capacity of 40 liters, RESUN LP20 water pump aerator, Internal Aquascape Filter Power and voltage: 120 V/60 Hz 5 Watt, PH-009(I)A pH meter to measure water pH, a DO meter DO9100–40 mg/L to measure dissolved oxygen, a PS-200A digital scale to measure carcass weight, 150 mm (6") digital caliper with an accuracy level of 0.01 mm, HTC-2 type thermometer for measuring temperature and a set of surgical tools photomicrograph microscope, conductivity meter, spectrophotometer, tissue cassette, incubator, rotary microtome, water bath, paraffin bath, and tissue processor.

The materials used in the study included 24 red tilapia with body weight of 7–9 g, TAKARI brand fish feed, PDAM water, physiological saline solution, 10% Neutral Buffered Formalin (BNF) fixative, xylol, graded alcohol (70, 80, 96%, and absolute alcohol), distilled water, paraffin, hematoxylin and eosin solution, enthelan.

The research design used was a 2 x 2 factorial Completely Randomized Design (CRD) with two main factors, each main factor consisting of two levels, the first main factor was an aerator and the second main factor was a filter. The aerator used is a single aerator and a double aerator, while the filter consists of a filter and without using a filter. Each treatment consisted of six replications so that each container contained six red tilapia fish. Feed is given every morning, afternoon and evening. The red tilapia used was 100 days old and treated for acclimatization for two weeks. After acclimatization, red tilapia were maintained with each treatment, namely single aerator without filter (ANF), double aerator without filter (AANF), single aerator with filter (AF), and double aerator with filter (AAF). Maintenance of red tilapia lasts for two months. Measurement of water parameters was carried out two times a week before the water in the container was replaced with new water.

Maintenance of red tilapia is carried out in plastic container boxes. The container used as a place for maintenance was previously washed with soap, then dried. Containers are placed in the room at the right position so that they get enough light. Acclimatization is carried out for two weeks so that the fish in the container can adapt to the new environment. Feed during acclimatization and research using the ad libitum method.

Fish kept for 60 days. Aerators and filters are used throughout the day and cleaned regularly. Feeding is carried out routinely 3 times a day, namely at 06,00 am; 01,00 pm; and 04,00 pm. The fish feed used is the Takari brand with a protein content of 31–33%. Water changes are carried out every two times in a week and previously measurements of the physical and chemical parameters of the water have been carried out. The physical and chemical parameters of water measured were dissolved oxygen, temperature, pH, salinity, ammonia, nitrate, and nitrite. Water change is done by draining 70% of the water in the container using a hose and replacing it with new water.

Carcass weight was obtained after the fish was killed, followed by the fish dissection stage by cutting and separating the non-carcass parts from the main part using a scalpel (dissecting set). The carcasses were weighed using a microbalance to record the results. Non-carcass parts such as the head, fins, internal organs of the fish and the tail can also be weighed using a micro balance. Skeletal muscle isolation can be done after weighing the carcasses. The muscles on the upper side of the fish body (epaxial area) were cut using a knife with a volume of 1 cm^3 so that the fixation process could take place perfectly. The muscle pieces are washed using physiological saline so that they are clean from dirt and blood, then they can be put into a sample bottle containing a fixative solution, namely 10% Neutral Buffered Formalin (NBF). The next step is making preparations using the paraffin method and staining with hematoxylin-eosin.

The diameter of the muscle fibers was calculated from the five selected fields of view. In each field of view, 5 muscle fibers were selected to measure their diameter. Measurements are made by drawing lines on the x-axis and y-axis so that the diameter of the muscle fiber can be calculated using the formula [(x-axis + y-axis)/2]. The number of muscle fibers was counted in the 5 selected fields of view. The structure of the muscle fibers was seen and documented to compare the histology of the muscles in each treatment. Observations were made using the help of a photomicrograph through magnifications of 400x and 1000x.

Analysis of data on carcass weight, muscle fiber diameter, and the number of muscle fibers was presented quantitatively, according to Susilawati did (2015) all data were analyzed using the SPSS. The observed data were first tested for distribution patterns using Shapiro Wilk and the homogeneity test using Levene Statistics. The data proves to be homogeneous ($P > 0.05$) and follows a normal distribution pattern ($P > 0.05$) then it continued using two way Anova.

RESULTS

The results of the analysis of carcass weight and muscle microscopic structure with different combinations of aeration and filtration are presented in Table 1, Figure 1, 2, and 3.

Table 1. Average carcass weight, non-carcass weight, muscle fiber diameter and number of muscle fibers after being given different aerator and filter combination treatments

Treatment	Carcass weight (g)	Non carcass weight (g)	Number muscle fiber	Diameter of muscle fiber (μm)
A and NF	13.82 ± 1.65 ^{a}	13.78 ± 4.47 ^{a}	41.3 ± 1.97 ^{a}	34.92 ± 1.80 ^{a}
AA and NF	21.72 ± 5.80 ^{b}	21.45 ± 6.65 ^{b}	47.9 ± 3.16 ^{b}	37.14 ± 2.13 ^{b}
A and F	27.25 ± 3.23 ^{c}	22.76 ± 5.16 ^{b}	50.93 ± 2.68 ^{c}	39.1 ± 2.08 ^{c}
AA and F	27.61 ± 3.87 ^{c}	26.23 ± 3.15 ^{c}	52.5 ± 1.92 ^{d}	44.79 ± 1.90 ^{d}

Notes: Data are presented as mean ± standard deviation. Means with different superscripts in same column showed significantly different (P <0.05). A (single aerator); AA (Double Aerator); NF (Non-filter); F (Filters)

Table 2. Increase body weight after being given different aerator and filter combination treatments

Treatment	Initial body weight (g)	Final body weight (g)	Increase body weight (g)	Percentage Increase body weight per day
A and NF	8.32 ± 1.65 ^{a}	27.60 ± 2.06 ^{a}	19.28 ± 1.37 ^{a}	3.86 ± 0.20 ^{a}
AA and NF	8.15 ± 2.20 ^{a}	43.17 ± 3.23 ^{b}	35.02 ± 1.16 ^{b}	7.16 ± 0.37 ^{b}
A and F	8.27 ± 3.23 ^{a}	50.01 ± 1.20 ^{b}	41.74 ± 2.68 ^{b}	8.41 ± 0.12 ^{b}
AA and F	7.70 ± 3.47 ^{a}	53.84 ± 3.51 ^{c}	45.64 ± 1.92 ^{c}	9.99 ± 0.18 ^{c}

Notes: Data are presented as mean ± standard deviation. Means with different superscripts in same column showed significantly different (P<0.05). A (single aerator); AA (Double Aerator); NF (Non-filter); F (Filters)

There was an interaction effect (P <0.05) between the use of aerators and filters on carcass weight, number of muscle fibers, and diameter of muscle fibers. However, non-carcass weight was not affected (P >0.05) by the interaction factor between the use of aerators and filters. Water quality in the double aerator and filter groups produces optimum DO, pH, and temperature values. Low concentrations of ammonia, nitrate, and nitrite are shown in Table 2 and 3.

Table 3. The value of dissolved oxygen, pH, water temperature, salinity, nitrite, nitrate, and ammonia in each treatment

Variable	Treatment			
	ANF	AANF	AF	AAF
D.O (mg/L)	5.59	6.74	7.36	8.46
pH	6.66	6.69	6.78	6.9
Water temperature (°C)	28.13	28.28	28.51	28.33
Salinity	0.1	0.1	0.1	0.1
Nitrite	0.198	0.128	0.045	0.042

Notes: ANF= group using one aerator without a filter, AANF= group using two aerators without a filter, AF= group using one aerator and using a filter, and AAF= group using two aerators and using a filter

Figure 1. Slices of red tilapia carcass

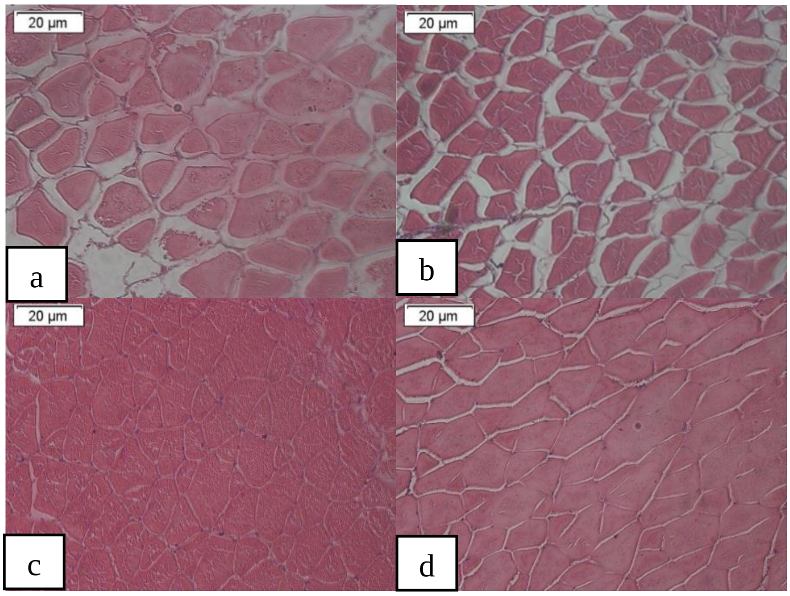

Figure 2. Histological structure of skeletal muscle fibers after aerator and filter administration, histological appearance in one field of view shows differences in the density of the number of muscle fibers in each treatment. ANF (single Aerator without filter) (a); AANF (unfiltered double aerator) (b); AF (single aerator with filter) (c); and AAF (dual aerator with filter) (d). (Stain HE, 400x magnification)

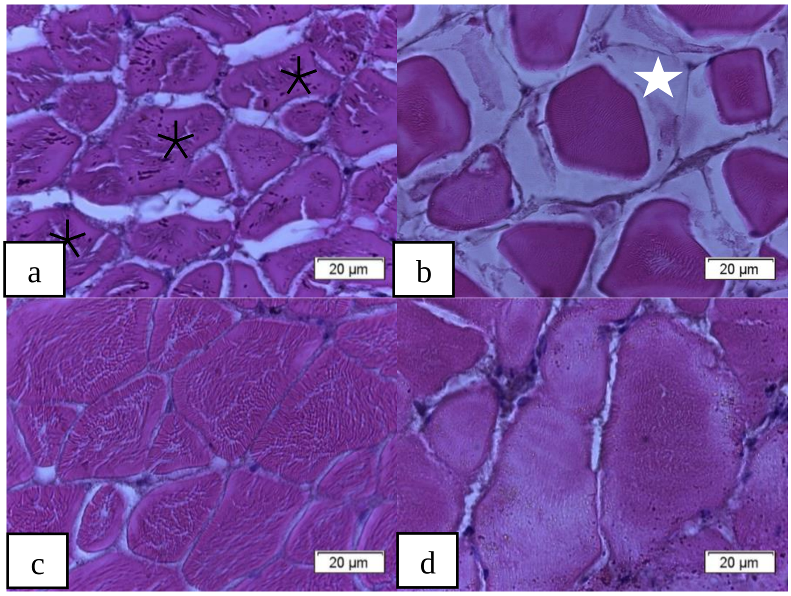

Figure 3. Histopathology of skeletal muscle fibers in fish given an unfiltered aerator, there is necrosis of the muscle fibers (black stars) and edema (white stars). ANF (single aerator without filter) (a); AANF (unfiltered double aerator) (b); AF (single Aerator with filter) (c); AAF (double aerator with filter) (d). (HE staining, 1000x)

DISCUSSION

The physical and chemical factors in the double aerator treatment equipped with a filter meet the requirements for fish life so that it can support fish growth. Fish growth can be optimal if the absorption, distribution, and utilization of nutrients take place without any disturbance so that the metabolic processes that occur in the body run smoothly. Metabolic products, both energy, and organic substances can be used to synthesize body tissue structures, including muscle synthesis, which in turn can be converted into meat. Zaidy and Tatty (2021) state that the quality of water and feed with a certain protein content used for cultivation can affect the quality of fish meat. Carcass composition, color, ratio, and growth are determinants of good or bad quality of fish meat produced.

Caring for fish by paying attention to water quality and fulfilling nutrition can have a good effect on muscle growth which is triggered by increasing fish body weight. Aerators and filters are used to meet water quality standards that can be accepted by fish so that the fulfillment of nutrients for metabolism and the energy produced can be used by fish for activities. Kartikayudha et al. (2013) stated that several factors such as activity and nutritional levels can affect the size of the diameter of muscle fibers and fatty tissue in meat. Muscles that are used for high activity will have a large diameter of muscle fibers. High activity will increase muscle contraction which in turn will result in hypertrophy so that there is an enlargement in the diameter of the muscle fibers. Knutsen et al. (2019) stated that fish can increase muscle mass in their bodies through hyperplastic and hypertrophic growth. The contribution of hyperplasia and hypertrophy in increasing muscle mass is influenced by several factors, including food and temperature.

The carcass cut as shown in Figure 1 shows that the carcass consists of edible parts of the fish body, so the head, tail, fins, bones, and organs inside the fish's body have been removed first. Table 1 shows that the treatment with two aerators and filters resulted in the removal of the non-carcass parts with the highest value and for the average carcass weight of fish treated with multiple aerators and filters also showed higher values. Treatment using a double aerator is able to increase the concentration of dissolved oxygen as an important component used by fish to carry out metabolism while the use of filters has an important role in filtering out impurities caused by food residue deposits and excrement produced by fish. Supporting the statement of Prinet et al. (2013) that the level of dissolved oxygen directly affects the metabolic rate, this can have an influence on the physiological conditions and performance of fish such as growth, activity levels and the ability of fish to process their food. Poor water quality can result in a decrease in appetite which will affect the conversion of feed to meat (Ajiboye et al., 2015).

Table 1 shows that the interaction between the use of aerators and filters has no significant effect on non-carcass weight. The size of the head, tail, fins and organs contained in the fish body follows the growth rate and life cycle of the fish. Water quality does not have a direct effect on the size of the non-carcass parts of the fish because the tail, fins and internal organs appear gradually following the stages or life cycle of the fish, especially when the fish are still in the larval form. Guaqiang et al. (2020) stated that when fish are in the initial developmental stage, namely larvae, organs in the body experience a faster growth rate than other parts until the organs have fully developed or reached a certain stage, changes in all parts of the fish will experience the same growth rate. following body mass.

Body weight has increased due to the addition of body muscle mass. Adequate nutrition is used for the process of protein metabolism which is important for the formation of muscles in the fish's body. Kartikayudha et al. (2013) stated that an increase in protein metabolism in the body will affect the formation of myofibrils. An increase in the number of myofibrils (hypertrophy) will result in a larger diameter of the muscle fibers. Myofibril protein is the main part in muscle tissue that plays a role in muscle contraction. The increase in the size and number of muscle fibers produced due to hypertrophy and hyperplastic occurred in all muscle fibers from each treatment, but the largest diameter values were found in fish treated with double aerators equipped with filters. As long as the fish is still in the larval stage then hyperplastic dominates for growth and if the fish has reached the juvenile to adult stage then hypertrophy changes which will dominate the growth of the fish. Research conducted by De Mello et al. (2016) showed that there is a positive relationship between muscle fiber diameter and body weight. The increase in the size of the diameter of the muscle fibers is related to the increasing number of muscle fibers. The number of muscle fibers in fish treated using a filter and two aerators had the highest value compared to the other three. This happens because the continuous formation of muscle fibers is accompanied by an increase in the diameter of the muscle fibers which is supported by optimum water quality parameters.

The density of the number of muscle fibers in each field of view in Figure 2, shows that the average number of muscle fibers in the single aerator treatment without a filter is lower and the distance between the muscle fibers is less dense as seen in the muscle fibers treated with a double aerator equipped with filter as shown in Table 1.

Growth is supported by the formation of muscle fibers with a very small diameter so that the muscle fibers in the field of view look very tight as a result of the formation of new myotubes or subsequently become new muscle fibers. Robisalmi et al. (2021) stated that the growth rate of fish directly depends on the accumulation of skeletal muscle mass which is rich in protein and amounts to more than half of the total body weight of fish. Johnston (2004) adds that 'hyperplastic' growth is an increase in the number of muscle fibers due to the formation of new muscle fibers. Formation of new myotubes and muscle fibers will continue until the fish has reached 40% of its maximum body length. Through hyperplastic growth, fusion between satellite cells forms new myotubes on the surface of preformed muscle fibers. In contrast to hyperplastic, through hypertrophy growth, satellite cells will activate an increase in the number of myonuclei and myofibril synthesis so that later the size of the diameter of the muscle fibers will continue to increase.

Figure 3 shows a comparison of muscle histology in each treatment. Muscle histopathology found in the maintenance treatment without being given a filter showed necrosis and edema. This is thought to occur due to the presence of toxic substances such as ammonia in high concentrations causing damage to fish muscles. Sabae and Mohammed (2015) stated that pollutants can cause histopathological changes in muscles, characterized by degeneration of muscle fibers and atrophy, focal necrosis and edema between muscle fibers, as well as vacuolar degeneration which causes muscle fibers to separate from one another. On the other hand, Shahid et al. (2021) said that the possibility of muscles being affected by pollutants is smaller than organs such as gills, this is due to the direct interaction that occurs between the gills and pollutants.

The values of ammonia, nitrate and nitrite in the treatment without a filter were higher than those in the treatment with a filter. These three compounds are able to affect the metabolism that occurs in the body so that all physiological processes as well as the formation of energy to increase muscle mass are disrupted. There are four main sources of nitrogen waste in aquaculture, namely fish excretion products, dead organisms, unconsumed feed residue and nitrogen gas originating from the atmosphere (Ciji & Akhtar, 2019). Hui-Wei and Michael (2016) stated that the main cause of loss of muscle mass and its function is influenced by excess ammonia values caused by a shift in metabolism from the liver to the muscles. Edema and necrosis are evidence of loss of function and muscle mass so that the muscle fibers do not have a perfect histological structure. Edema or swelling is caused by a buildup of fluid in the tissues, this causes the muscle tissue to look like it is spreading. The immune response from blood to tissues will be disrupted, causing lymphocyte infiltration and if it gets worse it will cause necrosis or death of muscle fibers (Aris & Tamrin, 2020).

CONCLUSION

Based on the results of the study it can be concluded that the use of multiple aerators equipped with filters does not cause damage to the muscle structure so that they can support fish growth in producing quality carcasses. Further research needs to be done using fish rearing media in natural ponds.

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