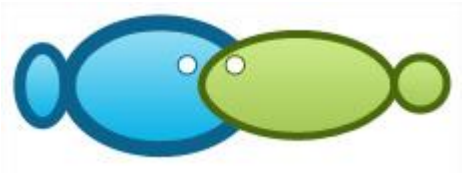

Hematology of Asian redtail catfish (*Hemibagrus nemurus*) in different stocking densities using an aquaponic recirculation system

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Abstract. Asian redtail catfish (*Hemibagrus nemurus*) is one of the economically important freshwater fish commodities in Indonesia and has been popular with domestic consumers in Southeast Asia. The high consumer demand for this fish incurs the intensification of cultivation, which is carried out with high stocking densities and high feed consumption. Determining the optimal value for stocking density is a prerequisite for ensuring growth performance, production, a lower stress level and fish health. Stress level and fish health can be seen from its hematology. This study aimed to obtain the optimal stocking density of redtail catfish for culture in a booster system using aquaponic recirculation. The optimal density was determined based on its hematology (erythrocytes, hematocrit, hemoglobin, leukocrit, and blood glucose). The research was conducted with a completely randomized design experimental method with 1 factor, 4 levels of treatment and 3 replications. The treatments consisted of different stocking densities: 600 fish m^{-3}, 700 fish m^{-3}, 800 fish m^{-3} and 900 fish m^{-3}. The redtail catfish fingerlings used had an average weight of 1.60±0.36 g per fish. Maintenance was carried out for 56 days. Analysis of blood images and blood glucose was carried out on day 1, day 28 and day 56. The results showed that the different stocking densities had a significant effect ($p<0.05$) on the hematocrit, hemoglobin, leukocrit and blood glucose values, but had no effect on erythrocytes and leucocytes values of redtail catfish. The best stocking density was 700 fish m^{-3}, with the number of erythrocytes of 2.56±0.05 x 10^6 cells mm^{-3}, a hematocrit of 31.3±1.5%, hemoglobin value of 10.53±0.47 g dL^{-1}, leucocytes value of 2.23±0.12 x 10^4 cells mm^{-3}, leukocrit of 1.80±0.10%, blood glucose of 63.33±3.51 mg dL^{-1}, and survival rate of 92.5±1.18%. The range of water quality during the study supported the life of redtail catfish. Temperatures ranged from 26 to 28.2^{0}C, pH was between 6-7.4, dissolved oxygen values were between 7-7.7 mg L^{-1} and ammonia values were between 0.0003-0.0257 mg L^{-1}.
Key Words: filter, fish health, survival rate, water quality.

Introduction. Redtail catfish (*Hemibagrus nemurus*) is one of the economically important freshwater fish commodities in Indonesia and has been popular with domestic consumers in Southeast Asia. Redtail catfish are native fish in inland waters that can live in lakes, rivers and swamps. In Indonesia, the fish inhabits the Kampar River in Riau Province (Husnah et al 2003), Barito River in Kalimantan (Samuel & Said 1995), Batanghari River in Jambi (Nurdawati et al 2006), Musi River in South Sumatra (Muflikhah et al 2006), Lake Singkarak in West Sumatra (Uslichah & Syandri 2003), among others.

The redtail catfish has a good meat quality, so the price is high compared to other freshwater fish (Boyoun et al 2010). This fish is preferred by the community because it is thick fleshed, slightly prickly and it has a delicious taste. The demand from Malaysia and Singapore is also high (Aryani et al 2002). The price of this fish can reach 5.15-5.49 USD kg^{-1} in fresh form, while for processed smoked fish the price is 17.17-27.47 USD kg^{-1}. However, commercialization and intensive farming of this species is still limited, due to the inability of the fish to breed naturally in captivity and slow growth; the provision of juvenile stages for rearing is still largely relying on capture from the wild (Roza et al 2014).

The high consumer demand for redtail catfish needs an intensification of cultivation, which is carried out with high stocking densities and large amounts of feed.

The application of booster system of fish culture with an aquaponic recirculation system can increase the production of redtail catfish. A booster system of fish culture is a super intensive culture system that applies a high stocking density technology using the application of a booster supplement product that contains nutrients, minerals and probiotics to improve water quality, feed and fish immunity. In order for the cultivation system to work optimally, it is necessary to have an appropriate stocking density of redtail catfish. Determining the optimal value for stocking density is a prerequisite for ensuring growth performance, production, low stress level and fish health. Stress level and fish health can be seen from its hematology. Hastuti & Subandiyono (2015) reported that African catfish (*Clarias gariepinus*) reared at high a stocking density (1.6 kg m^{-3}) showed a decrease in the number of erythrocytes, leukocrit, hemoglobin and haematocrit, which were below normal standards. However, there was an increase in the platelet count, which was above the normal range, indicating that the fish was under stress.

The information on high densities on the health of redtail catfish, especially on its blood profile is scarce. For this reason, this study was conducted with the aim of obtaining the optimal stocking density in the cultivation of the booster technique with the aquaponic recirculation system, verified with the blood profile.

Material and Method

Time and Location of the study. This research was conducted from March to September 2020 at the Hatchery Technical Implementation Unit and Experimental Pool of the Faculty of Fisheries and Marine Sciences, Riau University. The determination and interpretation of fish blood profiles was carried out at the Laboratory of Parasites and Fish Diseases, Faculty of Fisheries and Marine Sciences, Riau University, Pekanbaru.

Materials and instruments used. The materials used in this study were: 1000 redtail catfish juveniles with a weight ranging from 1.6±0.36 g per fish, which came from Kampar Regency. The test feed used was commercial pellet with 39% crude protein, 5% crude lipids, 16% ash, 12% moisture and 22% NFE, with the production code FF-999 from Charoen Pokphand Inc. The bok choy (*Brassica chinensis* L.) seeds used for the aquaponic system came from vegetable farmers on Kertama Street, Pekanbaru. Materials used for blood profile determination and interpretation were Hayem diluting fluid (1:200), Turk blood diluting liquid (1:20), 10% EDTA, 0.1 N HCl and distilled water. The tools used in this study were: a round tube test container with a diameter of 60 cm, a height of 45 cm, a volume of 100 L, an analytical scale with an accuracy level of 0.1 mg, a binocular microscope, a microhematocrit centrifuge model SH 120-1, a syringe, a hematocrit capillary, hematocrit reader, shelfs, Eppendorf tubes, gluco Dr, slides, cover glass, salinometer, crystoseal, hemocytometer, pH meter, DO meter, refractometer and spectrophotometer.

Experimental design. The research method used in this study was an experimental method using a completely randomized design (CRD) with 1 factor, 5 levels of treatment and 3 replications (Steel et al 1996). The variable in this study was the different stocking density. The treatment in this study referred to the best treatment obtained by Matondang *et al* (2019) in a booster system, namely the best stocking density of redtail catfish of 700 fish m^{-3} (1.12 kg m^{-3}). Thus, the treatments in this study consisted of: P1=600 fish m^{-3} (0.978 kg m^{-3}), P2=700 fish m^{-3} (1.169 kg m^{-3}), P3=800 fish m^{-3} (1.28 kg m^{-3}), and P4=900 fish m^{-3} (1.377 kg m^{-3}).

Preparation of test containers. The containers used in this study were 15 units of round plastic tubes with a diameter of 60 cm and a height of 45 cm, with a maximum water capacity of 100 L. Before use, the containers were washed using booster blue copper (each liter containing: 200 mg copper sulfate, 25 mg cetyl pyrinidium chloride, and 20 mg cetyl trimethyl ammonium bromide) at a dose of 1 ppm, and then dried. Next, the containers were filled with 80 L of water, and 1 ppm of booster blue copper was

added in order to conduct water sterilization (Wulandari 2014). The maintenance bath was equipped with a 32 watt pump to drain water from the maintenance container to the filter housing.

Preparation of bok choy plant seeds and filter containers. Bok choy seedlings preparation consisted in planting bok choy seedlings into rockwool measuring 3x3 cm^2, which had been perforated using a stick; each hole was filled one bok choy seedling (Zalukhu et al 2016). Seedlings used were not defective and were perfectly open, ranging between 4 and 4.5 cm in height, with 3 leaves each. Furthermore, each seedling was transferred to the netpot, which contained zeolite at the bottom, with rockwool on top. The amount of zeolite in each netpot was the same, 30 g. The zeolite was expected to purify water.

The assembly of the aquaponics system was conducted by making a hole in the maintenance container according to the size of the pipe, then connecting it to the gutter. The gutter used had 15 units with the size of 100x13.5x10.5 cm^3. Each gutter unit was made of 10 holes with a distance of 5 cm as a place for placing the plant netpot. Then the connection was made from each gutter using a paralon pipe to permit water flow.

The fish rearing container was equipped with a water pump with a power of 32 watts to drain the water into the fish tank. The filter tube used was a water gutter with a volume of 7 L. Furthermore, the water from the filter tube would flow through a PVC pipe with a diameter of 2.5 cm into the reservoir (plastic container with a volume of 10 L). The water collected in the reservoir was pumped into the maintenance tank with a pumping flow of 2 L per minute. Preliminary experiments were carried out to verify that the recirculation system for fish farming was working properly. In the preliminary experiment, the parameters observed were the rate of water change in the tank and in the filter (Putra & Pamukas 2011). After all the containers had been assembled, they were labelled according to treatment (Figure 1).

Figure 1. Container for fish rearing with the booster technique using the *Brassica chinensis* L. aquaponic recirculation system.

Preparation of media for fish farming. The growth of plankton in the redtail catfish rearing media was stimulated by adding a fermentation mixture made from 0.5 kg of bran, 200 cc of plankton booster, 10 g of aquaenzyme booster and 20 cc of liquid amino booster, incubated for 36 hours. The fermentation product was then dissolved in 10 L of water, and filtered, then 1.35 mL L^{-1} of suspension was put into the maintenance medium in the morning. This was repeated every 9 days (Pamukas et al 2020). Powerful Booster (Manstap with P_2O_5, KNO_3, SiO_2, and trace elements Ca, Mg, Co, Cu, Fe, Mn, Se, Zn) at a dose of 30 ppm was added in the afternoon after the distribution of the fermented booster suspension in the morning. The addition of water was performed after

administering the booster staple until the volume of water in the container reached 80 L (35 cm high).

Preparation of test fish. The criteria for the fingerlings selected as test animals were 1.6±0.36 g per fish in size, active, not defective, and relatively uniform in size. The fingerlings were stocked in densities according to treatments, converted to the volume of water in the containers: P1=600 fish m^{-3} (0.978 kg m^{-3}), P2=700 fish m^{-3} (1.169 kg m^{-3}), P3=800 fish m^{-3} (1.280 kg m^{-3}), and P4=900 fish m^{-3} (1.377 kg m^{-3}).

The fingerlings were soaked in a booster fish immunovit solution at a dose of 0.6 mL L^{-1} for 15 minutes. A stress off booster was applied with a dose of 2 mL for 5 L of water, then acclimatization was carried out by inserting a plastic bag containing the fish into the rearing container for 1-2 hours to adjust the water temperature.

Feeding. The frequency of feeding in this study was carried out 3 times a day, at 08.00, 13.00 and 16.00, with 3% of fish weight per day (Affandi et al 2009).

The supplements used from the first day to the 30^{th} day of maintenance were liquid amino booster 5 mL kg^{-1} of feed, booster grotop 2 g kg^{-1} of feed, and booster premix aquavita 2 g kg^{-1} of feed. Meanwhile, on the 31^{st} day until the end of maintenance, supplements used were liquid amino booster 5 mL kg^{-1} of feed, booster grotop 2 g kg^{-1} of feed, booster vitaliquid 2 g kg^{-1} of feed (Sudarmaji & Boster 2013).

Addition of supplements to feed was done by mixing all booster supplements according to the specified dose, then dissolved with water (100 mL kg^{-1}) and sprayed on the feed evenly and dried. In addition, the feed was stored in a clean and dry container. Each feed was weighed according to the body weight of the fish. The feed was given in an evenly distributed manner, so that each fish had equal access to food.

Maintenance. Redtail catfish were kept for 56 days. The length and weight of fish were determined every 14 days using an analytical balance with an accuracy of 0.01 and a gauge. Weight measurements were carried out to adjust the dose of feed administered. The survival rate was known by counting the number of fish dying each day during the rearing period. For 3 consecutive days after the fish were stocked and once a week during the maintenance period, multi-cell boosters were stocked at a dose of 20 ppm during the day.

Redtail catfish blood collection. Asian Redtail catfish blood sampling was conducted at the beginning of the study and after 56 days of maintenance. 3 fish samples were colelcted from each treatment and each replication. The observed hematology included the number of erythrocytes, hematocrit, hemoglobin, leukocrit, and blood glucose. The fish blood was collected by anesthetizing the test fish using clove oil (0.1 mL L^{-1}). The syringe and microtube used to draw blood were rinsed with 10% EDTA. Blood was taken using a 1 mL syringe from the caudal vein with a tilt angle of 45^0, the blood was inserted into a microtube (1 mL of blood could be collected), then put into a basin filled with ice cubes, so that the blood did not clot and was immediately observed in the laboratory.

Measured response. The parameters measured in this study were: total erythrocytes according to Klontz (1994), hematocrit and leukocrit levels according to Anderson & Siwicki (1995), hemoglobin level using the Sali method with a salinometer according to Wedemeyer & Yasutke (1977), total leukocrit according to Blaxhall & Daiesley (1973), blood glucose level according to Eames et al (2010), fish survival according to Effendie (1986), and water quality (temperature, pH, dissolved oxygen (DO), ammonia (NH_3), nitrite (NO_2), and nitrate (NO_3) were measured every week during the study according to Indonesian National Standard (SNI) in the Public Works Service (1990).

Data analysis. Data on total erythrocytes, level of hematocrit, leucocrit, hemoglobin, total leukocrit, blood glucose and fish survival during the study were analysed according to a completely randomized design model (Steel et al 1996). To determine the effect of the treatments on each measured variable, the analysis of diversity was carried out using

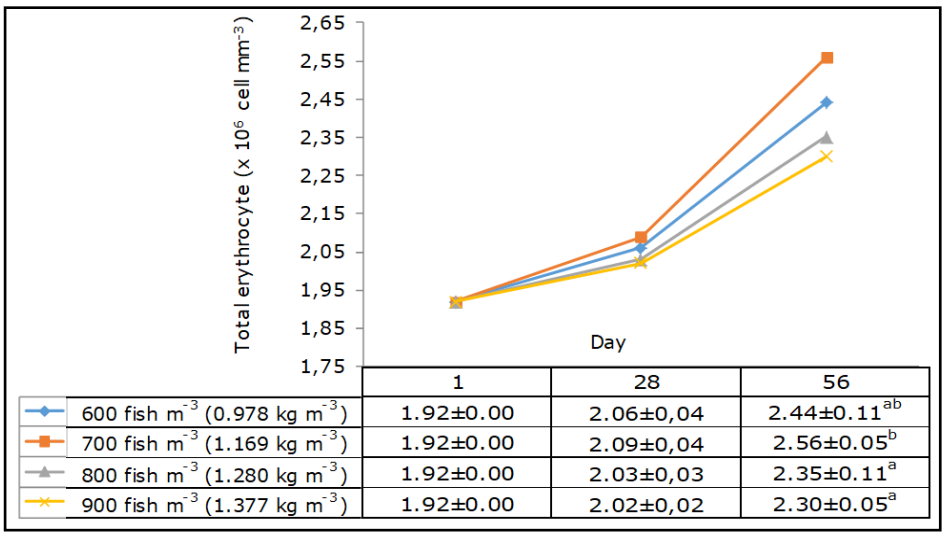
the F statistical test. The analysis process used SPSS version 13.0 software. If $p<0.05$, then the Newman-Keuls test was performed to see the differences among treatments. Water quality was analyzed descriptively.

Results and Discussion

Erythrocytes. The mean total erythrocytes at the beginning of the study (day 1) was 1.92 x 106 cells mm^{-3}. On day 28, the mean total erythrocytes increased in all treatments until day 56 (Figure 2). This was because the fish had adapted to the medium, so that stress level decreased and appetite increased, which was followed by an increase in erythrocytes. The highest increase in total erythrocytes was found at a stocking density of 700 fish m^{-3}, 2.09x106 cells mm^{-3} (day 28) and 2.56x106 cells mm^{-3} (day 56). This was because the stocking density was the optimal stocking density from the tested densities for redtail catfish, so that fish were in a comfortable condition and had an impact on improving health and ultimately increasing the number of erythrocytes.

Addini et al (2020) found that the number of erythrocytes in the stocking density of fish of 250 fish m^{-3} was greater than the number of erythrocytes at the stocking density of 200 fish m^{-3} and 300 fish m^{-3}. According to Harianto et al (2014), an increase in the erythrocyte level is caused by the physiological adaptation of the fish to the homeostatic conditions; the increased erythrocyte value indicates that the fish are in a healthy status. Erythrocyte levels were higher at the stocking density of 700 fish m^{-3} compared to 600 fish m^{-3}. It is assumed that the redtail catfish likes to school, so that the stocking density is suitable for the fish.

The total erythrocyte values at the stocking densities of 800 fish m^{-3} and 900 fish m^{-3} were lower compared to 700 fish m^{-3}. This is because at a high stocking density, fecal waste and metabolism will increase, so that the ammonia content will be higher than at lower stocking densities. This is in line with the opinion of Ni et al (2014), who stated that the number of erythrocytes is influenced by the density of distribution. The high stocking density caused the ammonia content in the culture medium to be higher. Furthermore, the performance of cells in the blood of African catfish was influenced by the quality of the water media, especially ammonia. According to Li et al (2013), catfish (*Pelteobagrus vachelli*) reared in high ammonia (5.7 mg L^{-1} TAN) contained a lower number of erythrocyte cells, hemoglobin and hematocrit compared to their values in fish raised in ambient ammonia (0.01 mg L^{-1} TAN). The erythrocyte value of *P. vachelli* cultured in high ammonia (5.7 mg L^{-1} TAN) was 2.28±0.07 ($x10^6$ cells μl^{-1}) and the catfish erythrocyte value in low ammonia was 2.7±0.08 ($x10^6$ cells μl^{-1}).



	1	28	56
600 fish m^{-3} (0.978 kg m^{-3})	1.92±0.00	2.06±0,04	2.44±0.11^{ab}
700 fish m^{-3} (1.169 kg m^{-3})	1.92±0.00	2.09±0,04	2.56±0.05^{b}
800 fish m^{-3} (1.280 kg m^{-3})	1.92±0.00	2.03±0,03	2.35±0.11^{a}
900 fish m^{-3} (1.377 kg m^{-3})	1.92±0.00	2.02±0,02	2.30±0.05^{a}

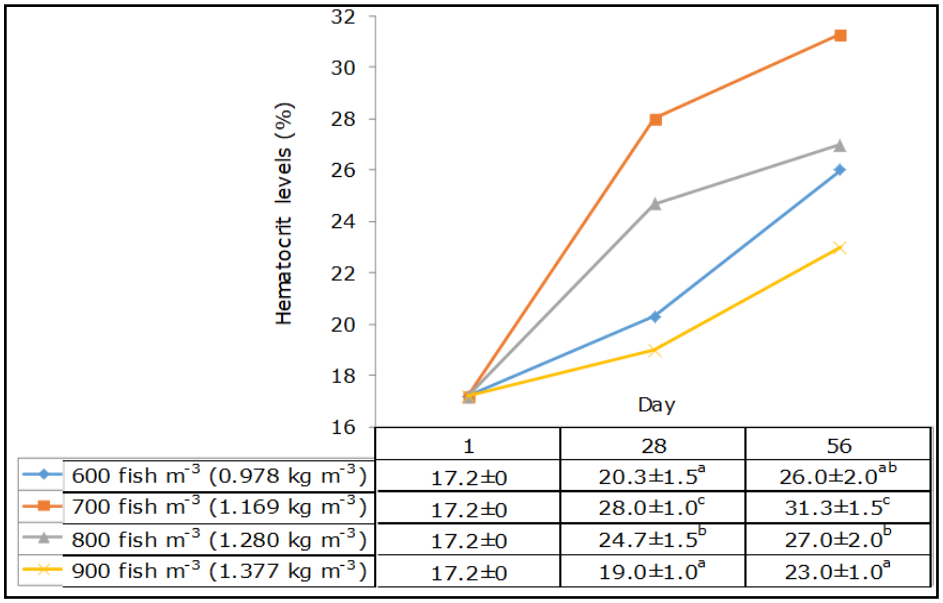
Figure 2. Average total erythrocytes at different stocking densities of fish.

The total erythrocyte level in all treatments during the study was in the normal range for the redtail catfish (1.92 - $2.56x10^6$ cells mm^{-3}). This is in accordance with the opinion of

Fitria et al (2019), who reported that the number of normal erythrocytes in redtail catfish living in their natural habitat in Ombilin and Sawahlunto were $2.268x10^6$ cells mm^{-3} and $2.267x10^6$ cells mm^{-3}, respectively. Furthermore, Irianto (2005) states that the number of normal erythrocytes in teleostei fish ranges from 1.05 to $3x10^6$ cells mm^{-3}.

The results of analysis of variance (ANOVA) showed that the stocking density had a significant effect ($p<0.05$) on the number of erythrocytes on day 56, while on days 1 and 28 there were no significant differences. The Newman-Keuls method (SNK) showed that the number of erythrocytes at a stocking density of 700 fish m^{-3} was different from the number of erythrocytes in other treatments on the 56th day. According to Emu (2010), the factors influencing the number of erythrocytes are species, sex, age, dietary nutrition and size. In addition, according to Yanto et al (2015), environmental conditions such as lack of oxygen and high temperatures can cause a decrease in the number of erythrocytes. In the study, although the test fish used were of the same age and size, and during culturing period the environmental conditions were relatively homogeneous, in the first 2 weeks the fish still adapted to the environment, feed and stocking density, so the number of erythrocytes did not increase significantly.

Hematocrit. The hematocrit level of redtail catfish increased from the beginning (day 1) to the end of the study (day 56). The highest increase in hematocrit occurred at a stocking density of 700 fish m^{-3}, while the lowest was found at stocking density of 900 fish m^{-3} (Figure 3). The hematocrit level is the ratio between red blood cells and blood plasma, so that the number is related to the number of erythrocytes (in our case depending on stocking density). Fitria et al (2019) state that a high number of erythrocytes will be followed by an increased percentage of hematocrit. According to Nuryati et al (2006), the hematocrit level is related to the number of red blood cells. Furthermore, Fadil et al (2011) state that an increase in the level hematocrit in fish blood indicates a relationship with the large number of red blood cells formed by the haematopoiesis tissue, the number of red blood cells being directly proportional to the hematocrit value. Setiawati et al (2017) explain that a low hematocrit count indicates a lack of food or decreased appetite due to stress.



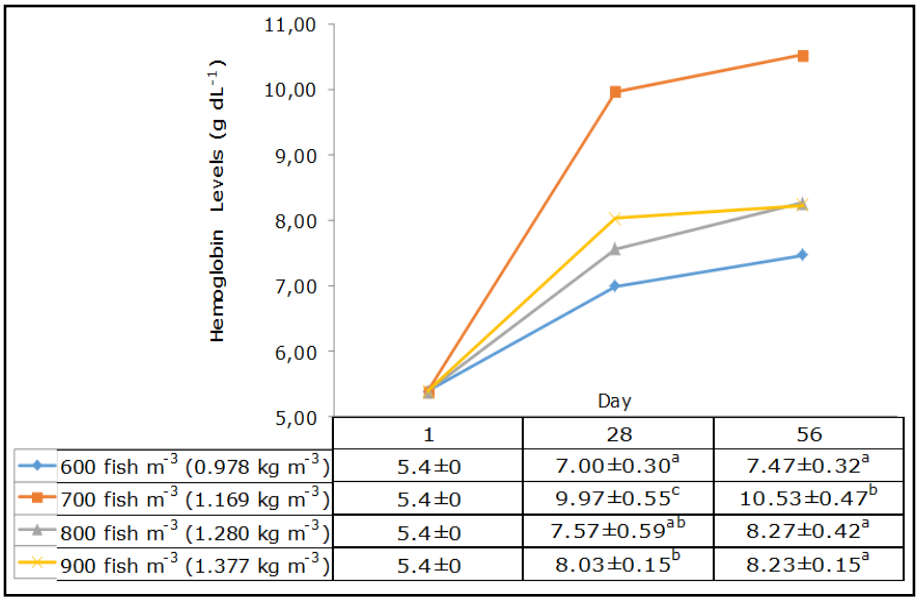
	1	28	56
600 fish m^{-3} (0.978 kg m^{-3})	17.2±0	20.3±1.5^{a}	26.0±2.0^{ab}
700 fish m^{-3} (1.169 kg m^{-3})	17.2±0	28.0±1.0^{c}	31.3±1.5^{c}
800 fish m^{-3} (1.280 kg m^{-3})	17.2±0	24.7±1.5^{b}	27.0±2.0^{b}
900 fish m^{-3} (1.377 kg m^{-3})	17.2±0	19.0±1.0^{a}	23.0±1.0^{a}

Figure 3. Average level of hematocrit at different stocking density of redtail catfish (*Hemibagrus nemurus*).

Based on the ANOVA test, the stocking density had an effect on the hematocrit level ($p<0.05$). The SNK test showed that the hematocrit value at the stocking density of 700 individuals m^{-3} was significantly different from the hematocrit value at the stocking densities of 600, 800 and 900 individuals m^{-3}. The hematocrit level of redtail catfish at

the beginning of the study was low, 17.2%, indicating that the fish were in the process of adapting to their environment. On day 28 and 56, the hematocrit levels increased to 19-31.3%. According to Bastiawan et al (2001), the hematocrit values in normal fish range from 30.8-45.5%. The hematocrit values at the stocking densities of 600, 800 and 900 fish m^{-3} were classified as low, presumably because the stocking density was not optimal, so that the fish were stressed and their appetite decreased. At the stocking density of 700 fish m^{-3}, the hematocrit value was classified as normal, indicating that the fish were healthy and the stocking density was optimal.

Hemoglobin. Hemoglobin levels in all treatments increased from baseline to the end of the study (day 56). The mean range of hemoglobin levels in the fish at the start of the study was low (5.4 g dL^{-1}), but on days 28 and 56 it increased within normal ranges (7-10.53 g dL^{-1}). According to Hardi et al (2011), normal hemoglobin levels in fish range from 6 to 13 g dL^{-1}. The increase in hemoglobin levels is due to the fish adapting well to the media. The use of the booster technique with aquaponic recirculation causes the water quality to be maintained in a good range for the life of the redtail catfish (Table 1), so that the fish is healthy, resulting in an increase in hemoglobin. According to Kurniawan (2019), hemoglobin levels in the blood are influenced by several factors, including water quality and oxygen content. DO in this study was classified as high, namely 7–7.7 mg L^{-1}.

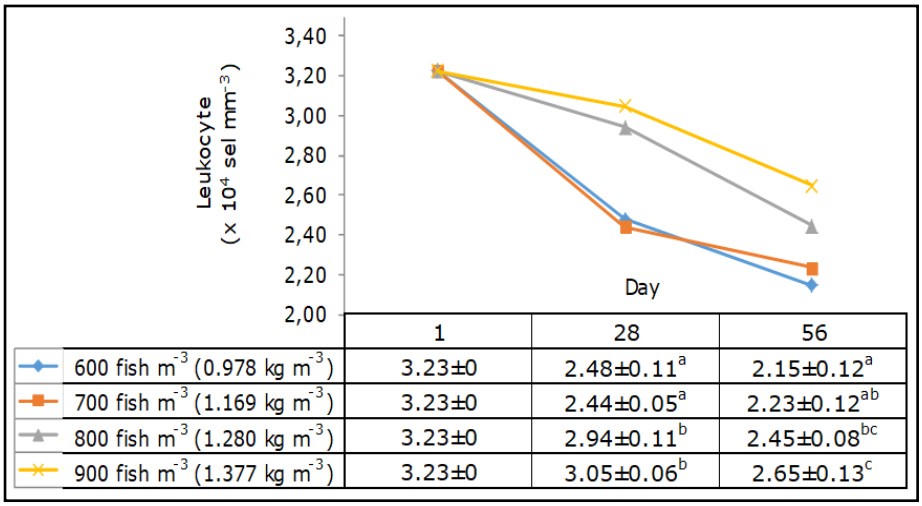


	1	28	56
600 fish m^{-3} (0.978 kg m^{-3})	5.4±0	7.00±0.30^{a}	7.47±0.32^{a}
700 fish m^{-3} (1.169 kg m^{-3})	5.4±0	9.97±0.55^{c}	10.53±0.47^{b}
800 fish m^{-3} (1.280 kg m^{-3})	5.4±0	7.57±0.59^{ab}	8.27±0.42^{a}
900 fish m^{-3} (1.377 kg m^{-3})	5.4±0	8.03±0.15^{b}	8.23±0.15^{a}

Figure 4. Average levels of hemoglobin at diffrent stocking densities of redtail catfish (*Hemibagrus nemurus*).

Figure 4 shows that the highest hemoglobin levels were found at the stocking density of 700 individuals m^{-3} and the lowest at 600 individuals m^{-3}. The ANOVA test results showed that the stocking density had an effect on the hemoglobin level of the fish ($p<0.05$). The SNK test showed that the hemoglobin level at the stocking density of 700 individuals m^{-3} was significantly different from the other treatments. This indicates that the stocking density of 700 fish m^{-3} is the optimal stocking density from the tested densities for redtail catfish. Caruso et al (2005) and Harianto et al (2014) stated that hemoglobin transports oxygen throughout the body. Hemoglobin releases oxygen to cells and binds to carbon dioxide. The amount of oxygen received by the tissue depends on the level and function of available hemoglobin. If the oxygen level is low, the ability of hemoglobin in blood transport will decrease. DO content during the study ranged from 7–7.7 mg L^{-1}, this value strongly supporting the growth of redtail catfish, because it is in the normal range of more than 3 ppm (Boyd 2015). The value of hemoglobin and oxygen levels produced in this study indicate that the red tail catfish are in a normal health status.

Leucocytes. The total leukocyte value during the study ranged from 2.15 to 3.32x10^4 cells mm^{-3} (Figure 5), which was in the normal range, which is 2-15x10^4 cells mm^{-3} (Royan et al 2014). The total leukocyte value in this study was similar to that obtained by Fitria et al (2019) from redtail catfish in their natural habitats, ranging from 2.7 to 3.5x10^4 cells mm^{-3}. This indicates the fish was in good health. During the study, the total leucocytes value of redtail catfish decreased from day 1 to 56. The decrease in total leukocytes was thought to be influenced by the production of anti-stress substances by probiotic organisms in the fish body, as reported by Mohapatra el al (2014). The decrease in total leukocytes during the study indicated a decrease in stress level due to fish being able to adapt to its environment. According to Fitria et al (2019), the total leukocyte value can be influenced by various factors, including water quality and pathogens in the maintenance media. The water quality during the study was in a good range for the health of redtail catfish due to the use of the aquaponic recirculation system booster technique in this study.



	1	28	56
600 fish m^{-3} (0.978 kg m^{-3})	3.23±0	2.48±0.11^a	2.15±0.12^a
700 fish m^{-3} (1.169 kg m^{-3})	3.23±0	2.44±0.05^a	2.23±0.12^{ab}
800 fish m^{-3} (1.280 kg m^{-3})	3.23±0	2.94±0.11^b	2.45±0.08^{bc}
900 fish m^{-3} (1.377 kg m^{-3})	3.23±0	3.05±0.06^b	2.65±0.13^c

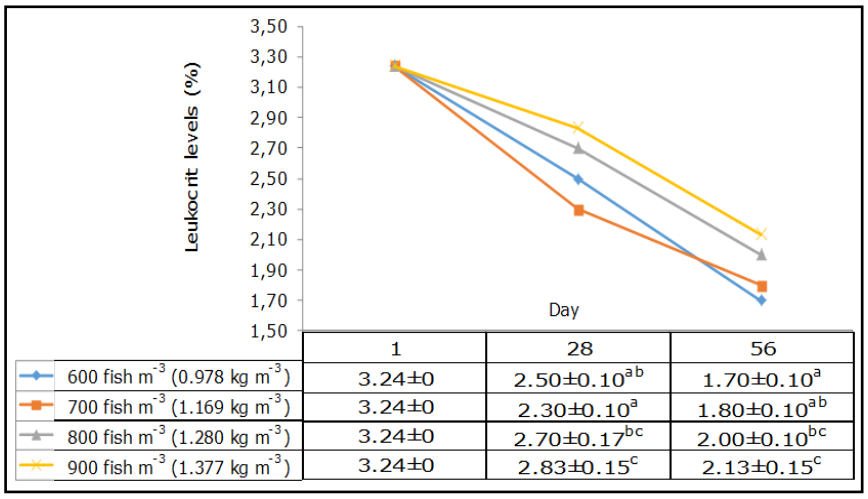
Figure 5. Average leukocyte levels at different stocking densities of redtail catfish (*Hemibagrus nemurus*).

The results of the analysis of variance (ANOVA) on total leukocyte count during the study showed an effect of stocking density on total leukocytes ($p<0.05$). Based on the SNK test, it was found that the total leukocyte at the stocking densities of 600 and 700 fish m^{-3} was relatively the same, but different compared to the stocking densities of 800 and 900 fish m^{-3}. The lowest total leukocyte value was found in the treatment with 600 fish m^{-3}, and the highest in the treatment with 900 fish m^{-3}. This indicates that at low stocking densities, the total leukocyte count tended to be lower. This is in line with the results of Caruso et al (2005) and Haenen et al (2010), who determined that the value of leukocytes tends to decrease along with the decreasing stocking density. This indicates that the fish did not experience phagocytosis or environmental stressors causing increased levels of leukocytes. The increase in total leukocyte count can be caused by diseases, infections, parasites, stress due to handling and environmental influence. A low leukocyte value indicates that the fish was in good health or had a good immune response.

Leukocrit. The leukocrit level decreased from the beginning to the end of the study. Leukocrit levels in all treatments were high (3.24%) at the beginning of the study, but decreased later, so that they reached the normal range (1.7 - 2.83%). The leukocrit level tended to decrease with decreasing stocking density of redtail catfish (Figure 6). Aisiah et al (2011) state that a normal leukocrit level ranges for catfish (*Pangasius hypophthalmus*) ranges from 0.72 to 2.89%. According to Hastuti (2007), a low leukocrit level can be caused by chronic infections, low nutritional quality, lack of vitamins and the presence of contaminants. Meanwhile, an increase in leukocrit can indicate an infection at

an early stage or that the fish is under stress. The leukocrit value of fish is also dependent on the condition of the fish at the time of sampling, the period of time between sampling and blood measurement, and the measurement procedure used. The decrease in leukocrit level indicates that the fish were not under stress and were not attacked by pathogens. The leukocrit levels were in the normal range.

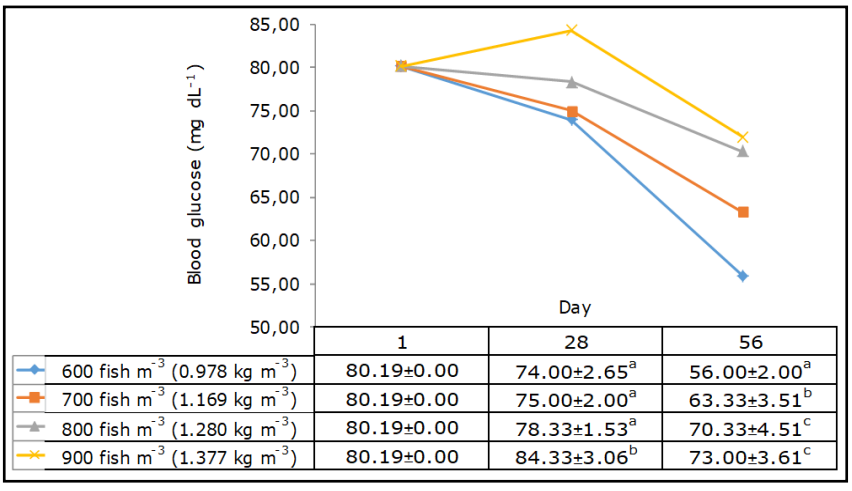
Based on the results of the analysis of variance (ANOVA), it was found that the different stocking densities had an effect on the leukocrit level of the fish ($p<0.05$). The leukocrit level in the stocking density of 700 fish m^{-3} was different from those in other stocking densities. This shows that the stocking density of 700 fish m^{-3} was the optimal stocking density for redtail catfish.



	1	28	56
600 fish m^{-3} (0.978 kg m^{-3})	3.24±0	2.50±0.10^{ab}	1.70±0.10^{a}
700 fish m^{-3} (1.169 kg m^{-3})	3.24±0	2.30±0.10^{a}	1.80±0.10^{ab}
800 fish m^{-3} (1.280 kg m^{-3})	3.24±0	2.70±0.17^{bc}	2.00±0.10^{bc}
900 fish m^{-3} (1.377 kg m^{-3})	3.24±0	2.83±0.15^{c}	2.13±0.15^{c}

Figure 6. Average leukocrit levels at different stocking densities of redtail catfish (*Hemibagrus nemurus*).

Blood glucose. The range of fish blood glucose decreased from day 1 to day 56, except in the stocking density of 900 fish m^{-3}, where it increased to day 28 and decreased to day 56 (Figure 7).



	1	28	56
600 fish m^{-3} (0.978 kg m^{-3})	80.19±0.00	74.00±2.65^{a}	56.00±2.00^{a}
700 fish m^{-3} (1.169 kg m^{-3})	80.19±0.00	75.00±2.00^{a}	63.33±3.51^{b}
800 fish m^{-3} (1.280 kg m^{-3})	80.19±0.00	78.33±1.53^{a}	70.33±4.51^{c}
900 fish m^{-3} (1.377 kg m^{-3})	80.19±0.00	84.33±3.06^{b}	73.00±3.61^{c}

Figure 7. Average blood glucose levels at different stocking densities of redtail catfish (*Hemibagrus nemurus*).

Figure 7 shows that the stocking density had an effect on the blood glucose level ($p<0.05$). Based on the SNK test, it was found that the blood glucose of the fish in the stocking densities of 600, 700 and 800 fish m^{-3} were relatively the same on the 28th day, but different from the stocking density of 900 fish m^{-3}. The blood glucose on day 56 differed between treatments, except in the stocking densities of 800 and 900 fish m^{-3},

where they were relatively the same. The blood glucose range in all stocking densities was within the normal range (56-84.33 mg dL^{-1}). Blood glucose levels decreased with the lower stocking density. This is in accordance with the research of Ajani et al (2015), who stated that the glucose level will increase when the fish experiences stress, directly proportional to the increase in stocking density used. According to Nasichah et al (2016), normal fish glucose level is between 40-90 mg dL^{-1}, whereas Hartanti et al (2013) give a different normal blood glucose in fish, between 41-150 mg dL^{-1}. Van de Nieuwegiessen et al (2009) reported that *Clarias gariepinus* responds to stress by increasing its blood glucose level. Putra et al (2020) state that stress hormones adrenaline and non-adrenaline are associated with cortisol secretion and glucose fluctuation to control energy use at high stocking densities.

Water quality. The temperature range, pH, DO and ammonia in all treatments during the study were relatively similar, except the highest ammonia concentration, which was found at the stocking density of 900 fish m^{-3} (Table 1). The water quality in all treatments was classified as good for the life of the redtail catfish. According to Tang (2003), the optimal temperature for the growth of redtail catfish is in the range of 27-33^{0}C. The optimal pH should be between 5.9-6.4, DO should be between 5.6-7.4 mg L^{-1} (Roza et al 2014), and ammonia should be below 0.02 mg L^{-1} (Saputra et al 2019). The water quality was maintained relatively constant during the study due to the application of the booster technique with aquaponic recirculation.

Table 1

Water quality for redtail catfish (*Hemibagrus nemurus*) at different stocking densities

Treatment	*Temperature (°C)*	*pH*	*DO (mg L^{-1})*	*Amonia (mg L^{-1})*
600 fish m^{-3} (0.978 kg m^{-3})	26.1–28.2	6–7.4	7–7.7	0.0003–0.0058
700 fish m^{-3} (1.169 kg m^{-3})	26.1–28.2	6–7,4	7–7.7	0.0003–0.0052
800 fish m^{-3} (1.280 kg m^{-3})	26.1–28.1	6–7.4	7–7.7	0.0003–0.0076
900 fish m^{-3} (1.377 kg m^{-3})	26–28.2	6–7.3	7–7.7	0.0003–0.0257

The survival rate. The average survival rate of redtail catfish in all treatments was high (79.9-92.52%). The highest survival rate was found at stocking density of 700 fish m^{-3} and the lowest was at the stocking density of 900 fish m^{-3} (Figure 8). The high survival rate at a stocking density of 700 fish m^{-3} occurred because the hematological conditions of the redtail catfish were at best values in this treatment compared to other treatments.

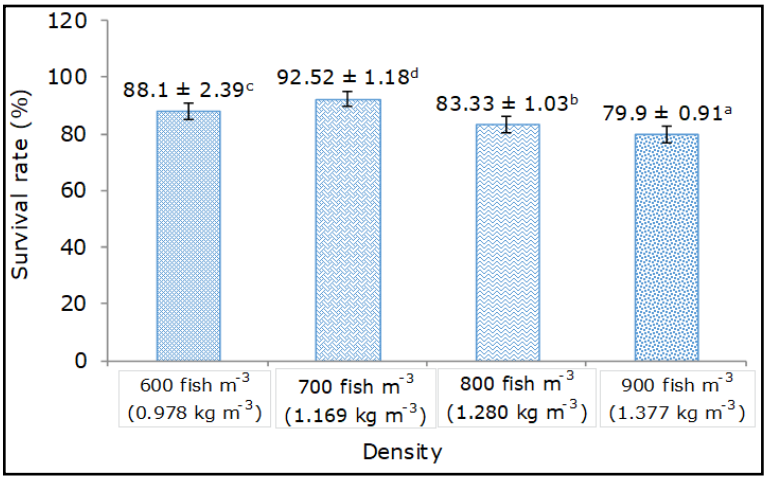


Figure 8. Fish survival rate at different stocking densities.

ANOVA results showed that the stocking density had an effect on the survival of the redtail catfish ($p<0.05$). Based on the SNK test, the survival rate was different among different stocking densities. This is because the hematological conditions of the redtail catfish were also different at different stocking densities. The survival rate in this treatment (1.169 kg m^{-3}) was higher than that obtained by Matondang (2019), which was 77.08-91.66%. This is due to the application of the aquaponic recirculation system booster technique, which caused water quality to remain in a good range for the growth and survival of the redtail catfish.

Conclusions. Redtail catfish stocking density affected total erythrocytes, hematocrit level, hemoglobin level, total leukocytes, leukocrit level, blood glucose and survival rate. The best stocking density was at 700 fish m^{-3} (1.169 kg m^{-3} – 5,096 kg m^{-3}), with the following values: number of erythrocytes: (2.56±0.05)x10^6 cells mm^{-3}; hematocrit: 31.3±1.5%; hemoglobin: 10.53±0.47 g dL^{-1}; leukocytes: (2.23±0.12)x10^4 cells mm^{-3}; leukocrit: 1.8±0.1%; blood glucose: 63.33±3.51 mg dL^{-1}; survival rate: 92.5±1.18%. The water quality during the study supported the life of redtail catfish.

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Conflict of Interest. The authors declare that there is no conflict of interest.

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