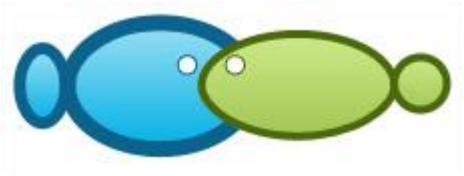

Aminotransferase, hematological indices and growth of tilapia (*Oreochomis niloticus*) reared in various stocking densities in aquaponic systems

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Abstract. Fish density is one of the important factors affecting growth and other physiological conditions. Hence, this paper aims to determine the influence of stocking density on serum glutamic enzymes, blood cells and biological performances of tilapia (*Oreochomis niloticus*) in aquaponic systems. The experiment was designed for 70 days and was conducted with the use of three plastic ponds of 2000 L in an aquaponic system. Each pond was divided in 4 cages of 0.5 m^3. Additionally, four stocking densities were used: treatment A - 50 fish m^{-2}; treatment B - 100 fish m^{-2}; treatment C - 150 fish m^{-2}; treatment D - 200 fish m^{-2}. The average weight of the fish was 5.90±0.09 g. The aspartate aminotransferase (AST) was measured at the end of the experiment with the following results: 91±13.11 U L^{-1}, 103.67±21.50 U L^{-1}, 98±10.15 U L^{-1} and 65.33±17.90 U L^{-1} in treatments A, B, C, and D, respectively. The volumes of alanine aminotransferase (ALT) in treatments A, B, C and D were 20.67±3.06 U L^{-1}, 20.33±4.73 U L^{-1}, 21.33±4.73 U L^{-1} and 18.67±6.11 U L^{-1}, respectively. The blood cell count and total bilirubin did not correlate with the stocking density. Blood glucose correlated with the stocking density. The highest blood glucose level was 73.00±12.53 mg dL^{-1}, in the stocking density of 200 fish m^{-2}. The highest number of leucocytes was 110.10±19.97x10^3 cell μl^{-1}, in the highest stocking density. Furthermore, the fish growth rate in treatment A was 2.89±0.07% day^{-1}, in B was 2.82±0.13% day^{-1}, in C was 2.58±0.07% day^{-1}, and 2.31±0.04% day^{-1} in D. The highest body weight was obtained in treatment A followed by B, C and D. The fish survival rate did not correlate with the stocking density and the water spinach plants were able to maintain good water quality parameters within the aquaponic system. The density of the tilapia cultivated in the aquaponic system has an effect on biological parameters, such as growth, feed consumption and feed utilization efficiency, but does not affect the survival rates and hematological performances.
Key Words: ALT, AST, bilirubin, blood cell, spinach.

Introduction. Tilapia (*Oreochomis niloticus*) has been labelled as the fish of the millennium considering the fact that it is highly used in aquaculture (Ada et al 2012; Andrei et al 2016; Budi et al 2017). The fish has the capacity to survive in almost all types of environment, is highly efficient in feed conversion, and exhibits fast growth rates. These are the traits that mark this species as ideal for aquaculture production. Although tilapia species are typical freshwater fish, they are known to tolerate a wide range of salinities. According to Jumah et al (2016), the fish has the ability to tolerate salinity conditions up to 30 ppt and this adaptation is associated with the increase in gill chloride cell density, size, enhanced activity of Na+/K+-ATPase, and a balanced level of Na+ and Cl- ions in the blood.

The aquaculture of tilapia is generally known as one of the food-producing sectors with the capacity of providing nutrients as well as safe food for humans. Hence, constant efforts are needed to improve the production in the industry (Budi et al 2017). According to Yarahmadi et al (2015), the stocking density is a key factor in determining the productivity and profitability of aquaculture systems production. The stocking density influences the growth rate, fish size and farm overall productivity (Rejeki et al 2013). In addition, according to Enache et al (2016) and Docan et al (2017), stocking density influences the health of farmed fish. Therefore, the stocking density has effects on the optimum use of water, on pond water quality, sedimentation rate, growth performance,

water productivity (Mohanty et al 2017) and fish mortality (Rejeki et al 2013; Andrei et al 2016). Rejeki et al (2013) reported that for tilapia culture in net cages at a density of 130 fish m^{-3}, the biological variables are in acceptable ranges, but begin to decrease at a density of 173 fish m^{-3}.

According to Yıldız & Bekcan (2017), the aquaponic system integrates aquaculture recirculating production processes with hydroponics, being environmentally friendly and sustainable. Some aquaponic systems use ipomoea plants (*Ipomoea aquatica*) for removing ammonia-nitrogen in it (Li et al 2007; Mchunu et al 2018). The ammonia-nitrogen buildup comes from the metabolism of feed, which is usually the second limiting factor for tilapia production levels, after dissolved oxygen. Therefore, there is a need for water quality management in order to maintain good water quality parameters. According to Sanchez et al (2019), a recirculating aquaculture system with tilapia is the most popular combination in aquaponics. Water rich in nutrients is considered a valuable resource for aquaponics. Plants cultured hydroponically absorb the nutrients excreted directly by the fish or generated by the microbial breakdown of organic wastes in the system. In addition, feeding and the level of stocking density of fish is directly related to the available nutrients in the system (Sanchez et al 2019; Yıldız & Bekcan 2017).

Water management strategies could be tailored to prevent the wasteful use of water, thereby enhancing its efficiency and productivity (Mohanty et al 2017). Hence, aquaponic is one of the fish culture systems with plants that could help maintain a good water quality. The design, technology and operational management of aquaponic systems is known to be overwhelmingly dependent on its stocking density. Considering the fact that a high density of fish usually produces high metabolic wastes, there is a need for a system with the capacity of removing these large amounts of metabolic wastes (Yarahmadi et al 2015). The aquaponic systems remove solid wastes and carbon dioxide, oxidize ammonia and nitrites, and aerate or oxygenate the water before returning to the culture tank.

According to Docan et al (2011), hematological indices are important parameters for the evaluation of the physiological status of fish. These hematological parameters respond quickly to changes in environmental conditions such as stocking density. In addition, blood cell and serum biochemical parameters of fish are useful tools for monitoring the health status of the fish, detecting illnesses and responses to rearing conditions. Erythrocyte, total leucocyte, hemoglobin, hematocyte and serum biochemical parameters like blood glucose (GLU), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and bilirubin are also vital parameters.

The glucose (GLU) is a permanent and immediate source of energy necessary for the operation of heart and muscles. The fastest and most cost efficient method of evaluating the stress condition of fish is through serial glicemy (Yarahmadi et al 2015). Density stress could also be the cause of marked elevations in serum glucose concentration. In addition, the nutritional condition could have an important influence on the glucose level. Hence, keeping the glucose within certain normal limits is one of the mechanisms with the finest homeostatic adjustment, to which the hepatopancreas participates, as well as some extrahepatic tissues and a series of endocrine glands (Patriche et al 2011).

ALT, formerly known as serum glutamic pyruvic transaminase (SGPT), is found mainly in the liver, but also in smaller amounts in the kidneys, heart, muscles, and pancreas. Usually, ALT is measured to know if the liver damage is associated with fish density stress (Jafarpour & Nekuie Fard 2016; Qiu et al 2016; Barton et al 2002). Normally, low levels of ALT are found in the blood, however, when the liver is damaged or diseased, it releases ALT into the bloodstream, thereby increasing its level (Price & Wilson 1995). Most increases in ALT levels are caused by liver damage and the ALT test is usually conducted along with other tests that check for liver damage, including aspartate aminotransferase (AST) and bilirubin (Hastuti et al 2019). Hence, this research focuses on these parameters.

An aspartate aminotransferase (AST) test measures the amount of this enzyme in the blood. AST, formerly known as serum glutamic oxaloacetic transaminase (SGOT), is normally found in red blood cells, liver, heart, muscle tissue, pancreas, and kidneys. Usually, low levels of AST are found in the blood but when a body tissue or an organ such

as the heart or liver is diseased or damaged, additional AST is released into the bloodstream. The amount of AST in the blood is directly related to the extent of the tissue damage (Dasgupta 2015). Additionally, after severe damage, AST levels rise within 6 to 10 hours and remain high for about 4 days. Both ALT and AST levels are reliable tests for liver damage and these are the two important amino transferases in the liver and mitochondria (Pratt 2010). Both are only found in low concentrations in the blood of healthy fish. According to Qiu et al (2016), increased ALT and AST activity in the blood serum is a reflection of liver damage and has been used as a biomarker for this damage and dysfunction.

Bilirubin is a byproduct of the routine destruction of red blood cells, which occurs in the liver (Hill 2009). It is normally released as bile in the feces and its elevation is a suggestion of liver dysfunction (Clayton 2009). Increased destruction of red blood cells has the capacity to cause elevated bilirubin levels even with normal liver function. The normal levels are between 0.1 to 1 mg dL^{-1}.

The aim of this study is to evaluate the effect of different stocking densities of tilapia (*Oreochromis niloticus*) reared in aquaponic systems on aminotransferase enzymes, blood cells and biological performances.

Material and Method

Experimental design. This study was conducted at the Aquaculture Laboratory, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, Diponegoro University, Semarang, Central Java, Indonesia. The study was conducted from June to November 2017. The experimental design contained a random group model (RBD) with four levels of stocking density, namely: A - 50 fish m^{-2}; B - 100 fish m^{-2}; C - 150 fish m^{-2}; D - 200 fish m^{-2}. Three groups of plastic pool containers were used, each partitioned with four nets, resulting in compartments measuring 0.5 m^3 (1x1x0.5 m). The containers were equipped with filters and water spinach plants (*Ipomoea aquatica*). In addition, aerators and pumps were installed in the containers. The aerators help to increase the dissolved oxygen content, while the pumps move water from the pond into the filters. Then, water is returned to the cultivation container pond after undergoing filtration and absorption by the water spinach.

Aquaponic biofilter system. In this research, 750 fish were used, with an average weight of 5.90±0.09 g. The selected test fish were placed into a test container prepared with an aquaponic recirculation system. During the study, the fish were administered feed containing 36.5% crude protein *ad libitum*, twice a day.

The filter materials were used to maintain the water quality during the study and include zeolite, pumice, water spinach, and bioballs. Water from the pond was allowed to flow into the filter tub using a pump. PVC gutters with dimensions of 2x0.1x0.1 m were used as the filter containers. There was also a hole at the base of the filter, helping in draining the water into the cultivation pond.

The zeolite used did not require special treatment and it had the ability to remove the ammonia from the water. The pore structure of the zeolite has sodium ions as a substitute for absorbed ammonia ions. According to Diansari et al (2013), the irregular zeolite crystal structure with a high surface area is a very effective trap for fine particulates and ammonia ions.

Bioballs were used for the growth of bacteria needed to maintain good water quality. The bioball media is light and easy to wash. Bioball filters maintain good water quality, with parameters in a reasonable range for fish growth.

Pumice was used as a growing medium for water spinach plants. The pumice contains lime $(CaOH)_2$, which is a source of Ca^{2+} ions that helps in stabilizing the pH of the water.

Blood sampling and analysis. About 2.5 mL of blood were sampled from 5 fish from each cage through caudal venous puncture using lithium heparin as anticoagulant at the end of the experimental trial. With routine methods used in fish hematology (Blaxhall &

Daisley 1973), the hematological indices were measured and analyzed. The red blood cell count (RBCc; 10^6 μL^{-1}) was determined by counting the erythrocytes from 5 small squares of Neubauer hemocytometer using Vulpian diluting solution. The hematocrit (PCV; %) was determined using heparinised capillary tubes centrifuged for 5 minutes at 12000 rpm in a micro hematocrit centrifuge. The photometrical cyanohaemoglobin method was used for determining the haemoglobin concentration (Hb; g dL^{-1}).

The ALT (E. C. 2. 6. 1. 2.) was assayed in a reaction mixture containing 65 mmol L^{-1} imidazol–HCl buffer (pH 7.4), 200 mmol L^{-1} L-alanine, 12 mmol L^{-1} α-ketoglutarate (α-KG), 0.15 mmol L^{-1} NADH, 0.025 mmol L^{-1} pyridoxal phosphate, 1 mmol L^{-1} EDTA, and 12 U L^{-1} lactate dehydrogenase (LDH, E. C. 1. 1. 1. 27). The AST (E. C. 2. 6. 1. 1) was assayed in a reaction mixture containing 65 mmol L^{-1} imidazol–HCl buffer (pH 7.4), 50 mmol L^{-1} L-aspartate, 10 mmol L^{-1} α-KG, 0.15 mmol L^{-1} NADH, 0.025 mmol L^{-1} pyridoxal phosphate, 1 mmol L^{-1} EDTA, and 8 U L^{-1} malate dehydrogenase (MDH, E. C. 1. 1. 1. 37) (Reitman & Frankel 1957; Wu 2002; Huang et al 2006; Wang et al 2016).

The total bilirubin was measured through the photometric method, which is a modification of the Jendrassik and Grof method (Jendrassik & Grof 1938; Ngashangva et al 2019).

Biological performance. The biological indicators calculated are: the total feed consumption (TFC), feed utilization efficiency (FUE), protein efficiency ratio (PER), relative growth rate (RGR), and the survival rate (SR), as shown below:

$TFC = F_0 - F_1$ (Pereira et al 2007)

Where: TFC - total amount of feed consumed (g); F_0 - initial amount of feed weight (g); F_1 - final amount of feed weight (g).

$$FUE = \frac{W_t - W_0}{F} \times 100$$ (Tacon 1987)

Where: FUE - feed utilization efficiency (%); W_t - total fish weight at the end of the experiment (g); W_0 - total fish weight at the beginning of the experiment (g); F - total amount of feed consumed (g).

$$PER = \frac{Wt - W_0}{Pi} \times 100$$ (Bake et al 2014)

Where: PER - protein efficiency ratio (%); W_t - total fish weight at the end of the experiment (g); W_0 - total fish weight at the beginning of the experiment (g); Pi - total amount of dietary protein consumed (g).

$$RGR = \frac{Wt - W_0}{W_0 \; x \; t} \times 100$$ (De Silva & Anderson 1995)

Where: RGR - relative growth rate (% day^{-1}); Wt - total fish weight at the end of the experiment (g); W_0 - total fish weight at the beginning of the experiment (g); t - rearing period of the experimental fish (days).

$$SR = \frac{\Sigma Lt1}{\Sigma Lt0} \times 100$$

Where: SR - survival rate of the experimental fish (%); $\Sigma Lt1$ - the total number of fish that survived at the end of the experiment; $\Sigma Lt0$ - the total number of fish at the beginning of the experiment.

Water spinach weight was monitored to establish its growth, being measured every two weeks.

Water quality. Observations on water quality include temperature, dissolved oxygen (DO), and acidity (pH). Measurements were carried out every two weeks. Observations on water quality consisting of ammonia (NH3), nitrite (NO_2) and nitrate (NO_3) content measurements were carried out at the beginning (week 0), mid-study (week 5) and at the end of the study (week 10).

Statistical data processing. The data was analyzed using SPSS version 21, involving One-way Analysis of Variance (ANOVA) tests. This was used to detect the differences between the aminotransferase enzyme concentrations, blood cell parameters, biological performance and stocking densities. The homogeneity of variances was tested with Levene's test. When the differences were significant at $P<0.05$ level, post-hoc Duncan's test was used to compare the means between the experimental variants.

Results and Discussion

Physiological parameters. The physiological responses to different stocking densities and their subsequent effects on aminotransferase enzyme, blood cell parameters and biological performance were assessed for Nile tilapia (Table 1).

Table 1

Physiological parameters of Nile tilapia (*Oreochromis niloticus*) reared in an aquaponic system in different stocking densities

No	*Physiological parameters*	*Stocking density in each treatment*			
		A (50 fish m^{-2})	*B (100 fish m^{-2})*	*C (150 fish m^{-2})*	*D (200 fish m^{-2})*
	Blood cell count				
1	Erythrocyte (x10^6 cell µL^{-1})	1.41±0.27^a	1.22±0.41^a	1.08±0.24^a	1.05±0.49^a
2	Total leucocyte (x10^3 sel µL^{-1})	87.57±20.83^a	91.27±4.25^a	103.37±21.26^a	110.10±19.98^a
3	Hemoglobin (g dL^{-1})	7.17±1.45^a	6.53±2.16^a	6.33±1.17^a	6.06±0.32^a
4	Hematocyte (%)	20.33±5.58^a	18.07±3.71^a	16.57±5.99^a	16.47±3.52^a
	Blood biochemistry				
5	Blood glucose level (mg dL^{-1})	26.67±1.41^C	29.33±4.62^b	38.00±5.29^b	73.00±12.53^a
6	ALT (SGPT) (Unit L^{-1})	20.67±3.06^a	20.33±4.73^a	21.33±2.08^a	18.67±6.11^a
7	AST (SGOT) (Unit L^{-1})	91.00±13.11^a	103.67±21.50^a	98.00±10.15^a	65.33±17.90^a
8	Total bilirubin (mg dl^{-1})	0.22±0.09^a	0.30±0.08^a	0.25±0.04^a	0.24±0.10^a

Note: different superscript letters show significant differences ($p<0.05$); ALT (SGPT) - alanine aminotransferase (serum glutamic pyruvic transaminase); AST (SGOT) - aspartate aminotransferase (serum glutamic oxaloacetic transaminase).

Based on the observed blood parameters, there were no significant changes in erythrocyte, total leucocyte, hemoglobin (Hb), and hematocyte levels. Similarly, the ALT, AST and total bilirubin levels showed no significant difference between treatment groups, while blood glucose level increased as fish stocking densities increased. The erythrocyte and red blood cell counts are important parameters that help maintain the functionality and integrity of the respiratory pigment. There were no changes in the number of red blood cells in different fish densities (Table 1).

Biological parameters. The results of the measurements of biological variables of tilapia cultivated in the aquaponic system with different densities are presented in Table 2. The stocking density of fish affects the biological responses such as growth, feed consumption,

feed utilization efficiency and protein efficiency ratio. The survival rate of the fish was not affected by stocking density.

The results show that individual growth processes in tilapia decrease with an increase in stocking density. Hence, the best individual fish growth was observed in the lowest stocking density of 50 fish m^{-2}. The individual weight gain of tilapia cultivated in the aquaponic system at various densities is presented in Figure 1. The higher the fish density is, the smaller the fish weight gain is.

Table 2

Biological parameters of Nile tilapia (*Oreochromis niloticus*) reared in an aquaponic system in different stocking densities

No	*Biological Parameters*	*Stocking density in each treatment*			
		A (50 fish m^{-2})	*B (100 fish m^{-2})*	*C (150 fish m^{-2})*	*D (200 fish m^{-2})*
1	Initial average body weight (g)	5.99±0.10^{a}	5.89±0.11^{a}	5.86±0.06^{a}	5.86±0.08^{a}
2	Final average body weight (g)	45.90±2.12^{a}	44.54±4.20^{a}	37.10±1.67^{b}	29.93±0.97^{c}
3	Initial biomass weight (g)	149.75±2.38	294.35±5.52	439.91±3.84	583.13±7.97
4	Final biomass weight (g)	1131.33±54.37	2121.33±174.47	2683.00±117.82	2933.00±133.61
5	Fish growth rate (% body weight day^{-1})	2.89±0.07^{a}	2.82±0.13^{b}	2.58 ±0.07^{c}	2.31±0.04^{d}
6	Feed consumption (g $fish^{-1}$)	33.65±1.99^{a}	29.85±1.89^{b}	29.63± 0.70^{b}	28.41±1.48^{b}
7	Food conversion ratio (FCR)	0.85±0.05^{ab}	0.80±0.06^{a}	0.99±0.04^{b}	1.20±0.02^{c}
8	Feed efficiency (%)	116.88±5.48^{b}	123.36±9.09^{a}	100.91±4.00^{c}	82.73±1.73^{d}
9	Protein efficiency ratio (%)	3.19±0.15^{b}	3.37±0.25^{a}	2.75±0.11^{c}	2.26±0.05^{d}
10	Survival rate (%)	98.67±2.31^{a}	95.33±1.15^{a}	96.44±2.04^{a}	93.00±5.20^{a}

Note: different superscript letters show significant differences ($p<0.05$).

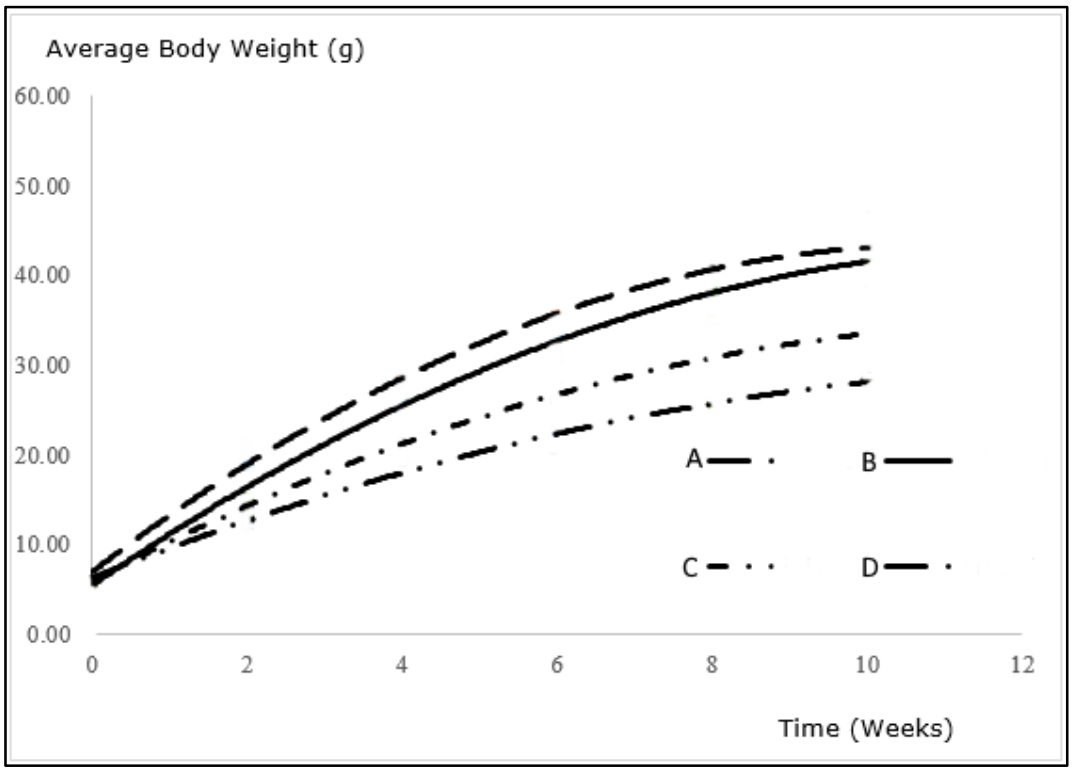

Figure 1. Average individual weight gain of Nile tilapia (*Oreochromis niloticus*) cultivated in the aquaponic system at various densities (A - 50 fish m^{-2}; B - 100 fish m^{-2}; C - 150 fish m^{-2}; D - 200 fish m^{-2}).

Water quality. The water quality parameters during the tilapia cultivation are presented in Table 3. Both the temperature and pH were stable during the cultivation, between 26.33 and 27.93°C and 7.54-7.85, respectively. The DO values were between 2.33 and 3.41 mg L^{-1}, which is the proper range for tilapia (Caldini at al 2011). The total ammonia concentrations were low, between 0.08 and 4.24 mg L^{-1}. This is an indication that the aquaponic biofilter system has succeeded in carrying out its role in breaking down ammonium nitrogen. Therefore, the NO_2 value is also low, between 0.05 and 0.31 mg L^{-1}, and the results of the breakdown, in the form of NO_3 are quite high, 5.50-15.91 mg L^{-1}, with the potential to be utilized by the water spinach.

Table 3

Water quality during Nile tilapia *(Oreochromis niloticus*) farming in the aquaponic system

Day	*Parameters*					
	Temperature (^0C)	*pH*	*DO (mg L^{-1})*	*NH_3 (mg L^{-1})*	*NO_2 (mg L^{-1})*	*NO_3 (mg L^{-1})*
0	27.93 ± 0.38	7.80 ±0.01	3.21±0.05	0.08±0.01	0.05±0.03	5.50±0.42
7	27.41 ± 0.13	7.54±0.09	2.92±0.10			
14	27.40 ± 0.12	7.48±0.04	2.42±0.42			
21	27.18 ± 0.11	7.54±0.06	2.61±0.03			
28	26.77 ± 0.19	7.61±0.02	2.49±0.09			
35	26.33 ± 0.08	7.54±0.03	2.59±0.04	1.16±0.22	0.16±0.04	7.29±1.01
42	27.00 ± 0.17	7.54±0.03	2.40±0.10			
49	26.77 ± 0.25	7.66±0.06	2.49±0.10			
56	27.10 ± 0.30	7.66±0.04	2.43±0.02			
63	26.70 ± 0.36	7.80±0.03	2.39±0.03			
70	26.70 0.20	7.85±0.03	2.33±0.01	4.24±0.67	0.31±0.05	15.91±0.59

Note: DO - dissolved oxygen.

The growth of water spinach. The weights of water spinach during the tilapia cultivation in the aquaponic system are presented in Figure 2. The model of the plant growth in quadratic form has the regression equation: $Y = -0.0763X^2 + 13.364X + 193.93$ and the coefficient of determination R^2 is 0.9978. Considering this equation, the highest weight of water spinach is predicted to be 779 g, on the 88th day.

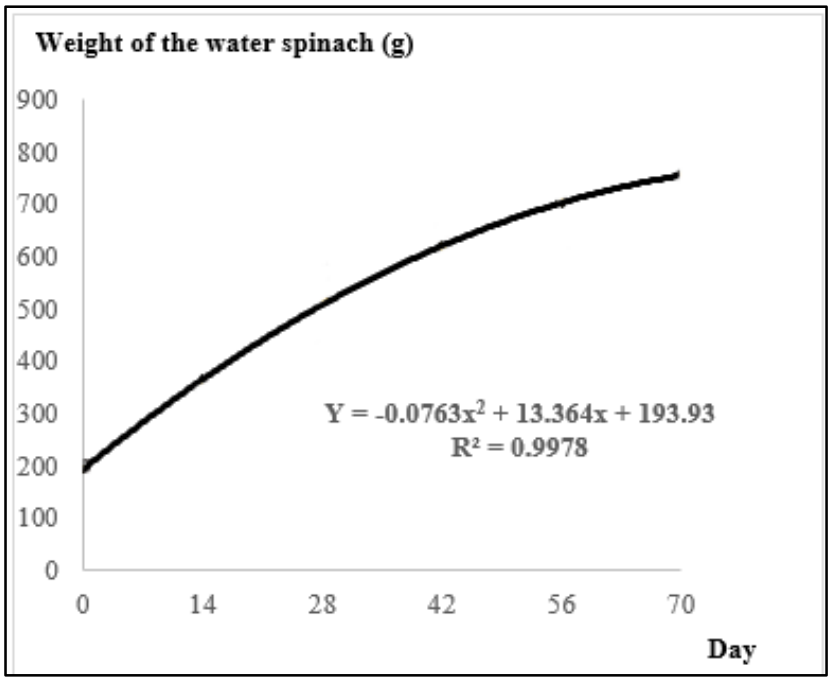

Figure 2. The growth of water spinach plant (*Ipomoea aquatica*) on biofilter media used in Nile tilapia (*Oreochromis niloticus*) fish farming system.

Four different stocking densities were applied and the tilapia from each were analyzed for physiological conditions and biological parameters. Water quality and the water spinach production were also determined in this study. Fish density is considered a chronic stress factor in an intensive aquaculture system, which could lead to physiological changes with negative effects on the fish biomass. This chronic stress can cause changes in the haematological parameters. Additionally, the physiological conditions of tilapia cultivated in the aquaponic biofilter system were in normal levels. The fish densities of up to 200 fish m^{-2} in the aquaponic system have no effect on the blood cell parameters and biochemistry, as presented in Table 1. The erythrocyte levels range from 1.05 to 1.41 (x 10^6 cells μL^{-1}), a value slightly lower than the value of erythrocyte levels found in tilapia by Ada et al (2012), which was 1.73±0.21 (x 10^6 cells μL^{-1}). However, the total leukocyte count found in this study, which ranges from 87.57 to 110.10 (x 10^3 cells μL^{-1}) was much higher than that found by Ada et al (2012), which was 2.77±0.06 (x 10^3 cell μL^{-1}). The hemoglobin and hematocyte values, as shown in Table 1, were higher than the values of the tilapia fish observed by Ada et al (2012), which were 5.33±0.65 mg dL^{-1} and 13.00±1.00%, respectively.

The density of tilapia in that culture system had an effect on the blood glucose levels. The blood glucose levels of fish fasted for 24 hours increase with increasing density. This blood glucose for this group was a result of the breakdown of glycogen from the liver. In these conditions, an increase in blood glucose levels illustrates the role of cortisol enzyme in the breakdown of glycogen into glucose. Cortisol is an enzyme that is an indicator of stress in fish. The fasting blood glucose values could as well indicate stress conditions. Table 1 shows the increase in blood glucose levels with increasing fish density. According to Morgan & Iwama (2011), serum glucose levels are considered a hormonal response to stress and are used as indicators of general stress. The results showed that glucose levels were significantly different between fish densities. A study conducted by

Patriche et al (2011) stated that fish blood values were significantly influenced by environmental conditions. The quality of water as shown in Figure 2, was appropriate for the tilapia culture, hence, the stocking density applied does not affect the blood cells variables.

In addition, some serum analyses are used to determine organ dysfunction, especially in the liver. According to Kesbiç (2019) and Sanchez et al (2019), the increase of ALT and AST levels in serum are indicators of liver dysfunction. Wan et al (2014) showed that for *Megalobrama amblycephala*, pH stress significantly influenced serum ALT levels. High serum transaminase activity was observed in tilapia in high density (more than 50 fish m^{-2}). These results showed that the serum ALT and AST levels of tilapia were not affected by the density of fish cultured in the aquaponic system. The ALT in the tilapia ranged from 18.67 to 21.33 U L^{-1}, while the AST ranged from 65.33 to 103.67 U L^{-1}. The ALT levels of the studied tilapia were higher than that of carp (*Cyprinus carpio*), which were around 5.40 to 7.49 U L^{-1} (Kesbiç 2019). Conversely, the ALT was lower than the 124.2 U L^{-1} found in catfish (*Clarias gariepinus*) (Hastuti at al 2019) and 44.51 U L^{-1} found in rainbow trout (*Oncorhynchus mykiss*) (Jafarpour & Nekuie Fard 2016). Furthermore, the AST results are lower than the AST values in sturgeon (*Acipenser baeri*) (Docan et al 2017), which was 491.8 to 609.4 U L^{-1}. Therefore, the density of tilapia up to 200 fish m^{-2} in the aquaponic system did not cause an increase in ALT and AST values. There was no suspected damage to liver cells of the tilapia fish. In addition, this condition is supported by the low bilirubin values. Hence, the fish density up to 200 fish m^{-2} in the aquaponic system produces healthy tilapia.

The tilapia cultivated in the aquaponic system with different stocking densities show different average individual weights, in which the higher density produces smaller individual weights. The tilapia used at the beginning of cultivation had the same weight. However, after being cultivated for 70 days, those with a density of 50 fish m^{-2} yielded the largest average individual weight, which was 45.90±2.12 g. In line with these results, a study conducted by Yıldız & Bekcan (2017) showed that fish density affected the fish growth parameters.

The water quality shows that the parameters are in appropriate range for tilapia culture. The survival rate was high, more than 93% and was not affected by the stocking density. It could be concluded that the aquaponic system was capable of supporting the life of tilapia with densities up to 200 fish m^{-2}.

The stocking density of tilapia cultivated in the aquaponic system has an effect on the final body weight, as well as on the biomass production, growth rate, feed consumption, feed conversion, feed utilization efficiency, and protein efficiency ratio. Tilapia with a density of 50 fish m^{-2} was considered as ideal for aquaculture production in a study conducted in India (Chakraborty et al 2010). According to Wu et al (2018), a high stocking density of tilapia results in decreased growth. The use of water spinach and filter materials in the aquaponic system was able to support good water quality. The growth of the water spinach was higher along with the increasing concentration of nitrate (NO_3), but limiting it causes the plant growth to decrease. Hence, reducing the growth rate of water spinach results in less absorption of nitrates, thereby accumulating its concentrations, as seen at the end of the study.

Conclusions. Fish density does not have significant effects on blood cell parameters, or blood biochemical performance parameters. However, it affects the blood glucose concentrations. It is found that the blood glucose increased along with the increase of fish density. The aquaponic cultivation system was able to improve the water quality to a level suitable for the life and growth tilapia. The density of the tilapia cultivated in the aquaponic system has an effect on biological parameters, such as growth, feed consumption and feed utilization efficiency, but does not affect the survival rates. The higher stocking density of the tilapia results in a smaller growth rate. The water spinach developed in line with the quadratic model ($Y = -0.0763X2 + 13.364X + 193.93$, with the determination value $R^2 = 0.9978$).

Acknowledgements. The authors wish to thank the nonPNBP research project, Diponegoro University, Indonesia, for funding this research.

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Received: 05 December 2019. Accepted: 07 December 2019. Published online: 05 April 2020.
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How to cite this article:
Hastuti S., Subandiyono S., 2020 Aminotransferase, hematological indices and growth of tilapia (*Oreochomis niloticus*) reared in various stocking densities in aquaponic systems. AACL Bioflux 13(2):813-824.