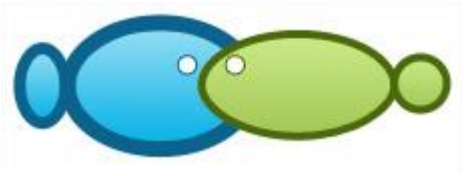

Haematological parameters of the North African catfish *Clarias gariepinus* farmed using biofloc technology

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Abstract. Application of biofloc technology (BFT) in fish culture is able to offer a solution to decrease environmental impact from fish cultivation activity. Biofloc is an aggregation of microbial communities consisting of phytoplankton, bacteria and organic particles. The biofloc technology converts toxic waste nitrogen into microbial proteins and helps to improve water quality without refreshing water. The aim of this study was to observe the haematological parameters of catfish (*Clarias gariepinus*) cultivated with density of 1,000 individuals m^{-3} using biofloc technology. With an explorative method, samples were taken from *C. gariepinus* cultivated with applications biofloc technology. Fish samples were observed every 2 weeks for 10 weeks of cultivation time. The measured variables were: total bilirubin, direct bilirubin, indirect bilirubin, and blood glucose. Blood plasma enzymes measured were ASAT and ALAT. Calculated blood cells are red blood cells, white blood cells, hemoglobin, hematocrite and platelets. Water quality measured included turbidity, conductivity, pH, DO, temperature and TAN. At the end of the study, the calculation of SR values was performed. The results showed that total bilirubin and indirect bilirubin were in normal ranges, while direct bilirubin values increased from 0.2 at week 6 to 0.5 mg dL^{-1} at week 8. According to the results *C. gariepinus* exhibits stress response with indicator of high blood glucose from 114 to 188 mg dL^{-1}. The concentration of aminotransferase enzyme, i.e. ASAT of 200 to 232 UL^{-1} and ALAT of 104 to 108 UL^{-1}. Blood cells consisting of red blood cells (1.6 to 1.7 million cells μL^{-1}), white blood cells (175 to 206.3 thousand cells μL^{-1}), hemoglobin (6.4 to 7.5 g dL^{-1}) hematocrite (21.7 to 26.2 %), platelets (1.1 to 9.3 thousand cells / µL). Water quality was in optimal condition which are in accordance with the *C. gariepinus* requirements with SR value of 95.7%.

Key Words: blood cell, aspartate aminotransferase enzyme, alanine transaminase enzyme, bilirubin, blood glucose.

Introduction. Bioflocs are a collection or aggregation of microbial communities such as phytoplankton, bacteria, and organic matter particles. Biofloc technology involves manipulation of the C/N ratio to convert toxic nitrogen waste into useful microbial proteins and helps in improving water quality under a system without refreshing water (Ahmad et al 2017). Biofloc function as a source of complete nutrition for aquatic organisms. Bioflocs together with some bioactive compounds will increase the growth, survival, and defense mechanisms of the fish body. Biofloc can be used for health management in aquaculture by stimulating the innate immune system of aquatic organisms. Advantages of microbial bacterial flocs and their derivatives such as organic acids, polyhydroxy acetate and polyhydroxy butyrate, can resist the growth of pathogenic bacteria (Ahmad et al 2017). Thus biofloc serves as a natural probiotic and immunostimulant.

Biofloc technology (BFT) has influence in aquaculture management, including water quality, feeding and disease control (Choo & Caipang 2015; Hapsari 2016; Ahmad et al 2017). The application BFT in aquaculture offers a solution to avoid the environmental impact of high nutrient release and to reduce the use of artificial feed. At BFT, the excess of nutrient waste in an aquaculture system is converted into microbial biomass, which can be consumed by cultivated fish and serves as a food source. This technology has ability to control its pathogens in aquatic environment. Researches on

Nile tilapia (*Oreochromis niloticus*) (Choo & Caipang 2015) and on white shrimp (*Litopenaeus vannamei*) (Effendi et al 2016) shows that the BFT can result in better production with lower feed conversion rates, good nutrition, water quality and optimal health (Choo & Caipang 2015; Effendi et al 2016; Nurhatijah et al 2016).

Application of biofloc technology in *Clarias gariepinus* culture has been done by Hapsari (2016). In that study, cultivation of *C. gariepinus* was performed by applying the biofloc system using a density of 1,000 individuals m^{-3}. The results showed that water quality during the experiment had stable values: average temperature 28.7°C, pH of 7.4 to 7.8, ammonia of 2.01 to 2.25 mg L^{-1}, nitrites of 0.1 to 0.4 mg L^{-1}, nitrates of 37.3 up to 38.1 mgL^{-1}. The growth of *C. gariepinus* reached 0.30±0.06 g/day, while the recorded feed conversion value was 0.81 to 0.95.

The application of biofloc technology in *C. gariepinus* culture shows that probiotics are used as alternative feed and the addition of molasses in probiotic fermentation can increase the nutritional value of biofloc so as increase the growth of the species. The floc density in the BFT does not affect the salinity, temperature, pH, DO, TAN and nitrite concentrations, but affects the concentration of nitrates and alkalinity (Nurhatijah et al 2016). Research on biofloc application on *C. gariepinus* culture has only evaluated the growth, feed conversion and water quality (Hapsari 2016). The BFT study on *C. gariepinus* culture with very high density on fish physiology and haematological parameters is not yet known. Therefore, it is important to conduct a study that examines physiological condition and the haematological parameters of the species.

The *C. gariepinus* is known as an important source of protein of fish worldwide (Muchlisin et al 2014). The *C. gariepinus* is a native African species, which is very popular and is commonly cultivated in Indonesia. *C. gariepinus* has fast growth, and it is utilized for a wide variety of foods. This specie is able to tolerate various conditions of water quality and can be cultivated at very high density; therefore *C. gariepinus* have a great potential to be developed.

Intensive *C. gariepinus* farming system will be able to increase production significantly; however will have an impact on the environmental pollution. Therefore, the aquaculture industry requires control or control methods to contribute to the reduction of the environmental pollution and environmental degradation. For that sustainable and environmentally friendly *C. gariepinus* cultivation system is required. One of the eco-friendly aquaculture technologies is the Biofloc Technology (BFT) (Choo & Caipang 2015; Ahmad et al 2017).

The biofloc technology is useful in maintaining optimum water quality parameters within a system without water exchange. This technology is able to prevent eutrophication and waste disposal into environment (Ahmad et al 2017). Moreover, its technology will be useful to ensure biosecurity, as there is no water exchange except mud removal. This technology is economically feasible, environmentally friendly, and socially acceptable.

The basic principles and mechanisms of BFT are known; however, this technology requires justification and in its implementation requires further intensive research to make it sustainable in the future. The effect of biofloc technology application with very high fish density on haematological parameters and *C. gariepinus* health performance needs to be studied. This research is important to do to obtain justification of physiological condition of *C. gariepinus* cultivated with the BFT technology.

Hematological parameters are important indicators for evaluating the physiological status of fish (Docan et al 2011a). Physiological stress caused by application of aquaculture technology significantly affect red blood cells (RWB), hemoglobin, haematocrite and total white blood cell (WBC) of cultured fish (Docan et al 2011a). Hemoglobin as a haematological indicator is influenced by fish stocking density (Docan et al 2011b).

This study aims to observe the blood performance of *C. gariepinus* cultivated with high density using biofloc technology.

Material and Method. This research was conducted at Pemalang, Central Java, Indonesia, using explorative method. Fish samples were *C. gariepinus* cultivated using

biofloc technology, at stocking densities of 1,000 individuals m^{-3} without water change. The blood chemistry variable was measured from the fish blood sample of 10 fish. Fish sampling was done every two weeks for 10 weeks of fish farming. At the end of the study, the fish survival was evaluated.

Blood chemistry variables measured includes aminotransferase enzyme concentrations of ALAT and ASAT, blood glucose, total bilirubin, direct and indirect bilirubin. The calculated blood cell variables consisted of red blood cells (RBC), total white blood cell (WBC), hemoglobin, hematocrit, platelets. The biological variable measured was fish survival. Water quality parameters consisting of dissolved oxygen, temperature, pH, conductivity and turbidity were measured using the Horiba's water quality checker tool. Total ammonia (TAN) was measured with ammonia test kit Microquant 114750, merck, Germany. The measurement of water quality parameters was done every 2 weeks during the experiment.

The glutamate pyruvate transaminase enzyme (ALAT) was measured using Photometric-UV test (GPT/ALAT, EC2.6.1.1) and glucamate oxaloacetate transaminase (ASAT) serum blood of *C. gariepinus* measured by Photometric-UV test (GOT/ASAT, EC 2.6.1.1). Total bilirubin and direct bilirubin were measured by the method of photometric test modification of the Jendrassik/Gróf method. *C. gariepinus* blood cells were measured by the ABX hematology analyzer. Blood glucose was measured with blood glucose test kit (GLUCOCARD Test Strip II, ARKRAY, Japan). The data's were analyzed descriptively, and compared to the normal value and value of reference according to Jendrassik & Grof (1938).

Results and Discussion. Blood characteristics can be used to evaluate physiological responses in fish. The measurements of various blood cell and blood chemistry variables of *C. gariepinus* cultivated with biofloc technology, at stocking density of 1,000 individuals $m^{-3}$with no water change are presented in Figures 1, 2 and 3. Figure 1 shows that total bilirubin and indirect bilirubin of *C. gariepinus* during cultivation period by using biofloc technology is still within normal range. The value of bilirubin is higher than the value of bilirubin of fish cultivated using water flow system at stocking density of 200 individuals m^{-3} (Hastuti & Subandiyono 2013). Direct bilirubin of *C. gariepinus* cultivated using biofloc technology has increased over time during the cultivation period (Figure 1b). Direct bilirubin values at week 8 (0.5 mg dL^{-1}) and week 10 (0.3 mg dL^{-1}) are above normal level. The value of bilirubin is higher than the value of *C. gariepinus* bilirubin cultivated in flowing water system (0.17±0.10 mg dL^{-1}) (Hastuti & Subandiyono 2013).

Bilirubin is a yellow pigment derived from the breaking of heme. Heme is part of hemoglobin. The process of splitting hemoglobin in red blood cells (RBC) is performed by reticuloendothelial cells. Reticuloendothelial cells produce insoluble bilirubin in water; the secreted bilirubin in the blood is attached to albumin to be transported in the plasma to the liver. In the liver, hepatocytes release the bond and conjugate it with glucoronic acid so that it is water soluble. This conjugation process involves the glucoronyltransferase enzyme. Bilirubin will be transported to the liver and collected in the gallbladder. Bile is used in the process of digesting fats. In laboratory tests, bilirubin is examined as total bilirubin and direct bilirubin. While indirect bilirubin is calculated from the difference of the total and direct bilirubin. The measurement method used is photometry or spectrophotometry that measures the intensity of azobilirubin color.

Direct bilirubin is a bilirubin bound to albumin and insoluble in water. The normal value of direct bilirubin is ≤0.25 g dL^{-1} (Jendrassik & Grof 1938). Indirect bilirubin is a direct bilirubin that has been released from its bond with albumin and will bind to glucuronic acid. The process of breaking a bilirubin bond occurs in the liver. All of this indirect bilirubin will be collected in the gallbladder and under normal circumstances not present in the blood plasma. Indirect bilirubin is soluble in water.

The high concentration of direct billirubin is due to high heme destruction. In the present study, value of direct bilirubin of *C. gariepinus* was 0.5 mg dL^{-1} at the 8^{th} week and 0.3 mg dL^{-1} at week 10. This condition indicates that *C. gariepinus* cultivated in high density and BFT application has increased heme destruction in blood. It can also be seen from the research that the value of hemoglobin (Hb) and red blood cell (RBC) of *C.*

gariepinus were low (Figure 3 a & c). Furthermore, the value of indirect billirubin was 0.2 to 0.4 mg dL^{-1} and according to (Jendrassik & Grof 1938) it is considered under normal conditions (≤0.75 mg dL^{-1}). The value of the direct bilirubin was high, while the indirect billirubin value was within normal limits. That was indicating that the liver cells are working hard to release the bilirubin bond with albumin and replace its bond by glucoric acid. The hard work of the liver cells can also be seen from the enzyme aminotranferase, namely ASAT and ALAT (Figure 2 a & b). *C. gariepinus* is also in a stressful condition (based on the value of blood glucose in the Figure 1 d).

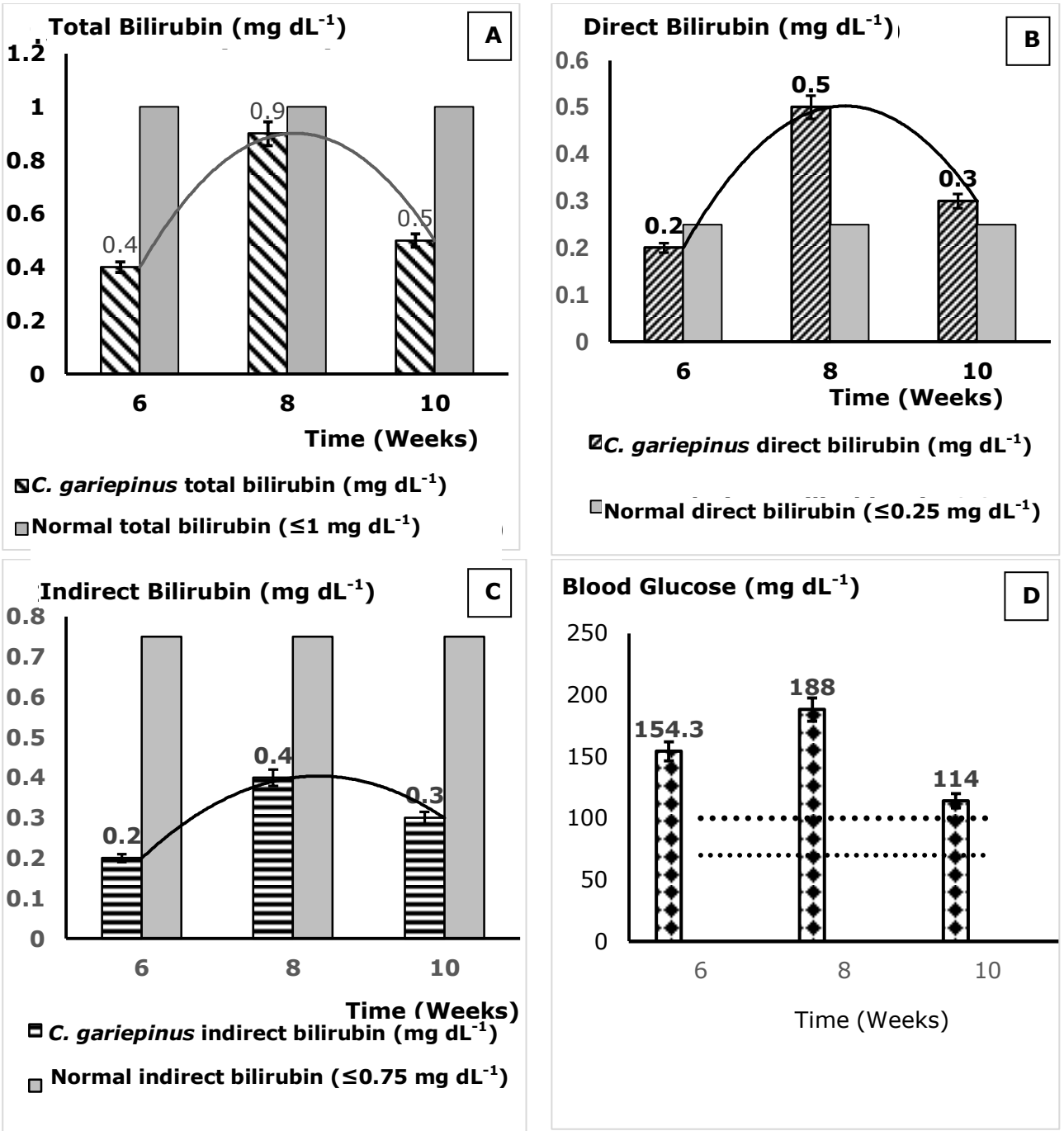

Figure 1. Bilirubin and blood glucose level of *Clarias gariepinus* reared with biofloc technology.

Based on Van de Nieuwegiessen et al (2009), *C. gariepinus* respond to stress by showing increased blood glucose levels. The value of blood glucose of *C. gariepinus* cultivated with high density and BFT applications is 114 to 188 mg dL^{-1} (Figure 1d). The value is high and above the normal blood glucose level (i.e., 80 to 100 mg dL^{-1}). The blood glucose

value is also higher than the blood glucose value of *C. gariepinus* cultivated with a density of 200 individuals m^{-3} using a flowing water system (75.33±4.70 mg dL^{-1}) (Hastuti & Subandiyono 2013). The high blood glucose values indicate that *C. gariepinus* cultivated with a density of 1,000 individuals m^{-3} using BFT system is under stressful conditions.

Stress is a defensive response of fish against the cause of stress (stressor). Various sources of stress form of environmental factors (temperature, salinity, pH, light, cultivation system, fish density) will have a negative impact on the physiological changes of the fish body. These changes include growth disorders, productivity and all activities as a result of disrupted homeostatic mechanisms in the body.

The aspartate aminotransferase (ASAT) enzyme, also known as serum glutamate oxaloacetate transaminase (SGOT), is a mitochondrial enzyme that serves to catalyze the transfer of alternating amino groups from aspartic acid to α-oxaloacetic acid to form glutamic acid and oxaloacetate (Price & Wilson 1995). The enzyme alanine aminotransferase (ALAT) is also referred as serum glutamate pyruvate transaminase (SGPT). Changes in liver enzyme values i.e. ASAT and ALAT can be studied to determine the liver organ disorders (Jafarpour & Nekuie 2016).

The aminotransferase enzyme is an enzyme that catalyzes the transamination reaction. There are two types of serum transaminase enzymes namely aspartate aminotransferase (ASAT) enzyme and alanine aminotranferase (ALAT) enzyme. The main source of ASAT enzyme is in liver, whereas ALAT enzyme found in tissues especially in heart, skeletal muscle, kidney and brain (Cahyono & Suharjo 2009).

Normal values of ASAT levels are <35 U L^{-1} and ALAT is <41 U L^{-1} (Pratt 2010). The higher ASAT and ALAT enzyme values, indicates increasing the rate of liver cells damage (Cahyono & Suharjo 2009). The concentration of ASAT enzymes in *C. gariepinus* cultivated using biofloc technology (BFT) is 200 to 232 U L^{-1} (Figure a). The high value of ASAT enzyme indicating liver cell damage (Cahyono & Suharjo 2009; Jafarpour & Nekuie 2016). The ASAT value is also higher than ASAT value in *C. gariepinus* cultivated with density of 200 individuals m^{-3} and water flow, that is equal to 185.33±5 U L^{-1} (Hastuti & Subandiyono 2013). The high value of ASAT in *C. gariepinus* cultivated using biofloc technology is suspected to be due because the fish liver cells work hard due to stress conditions caused by the high density of fish of 1,000 individuals m^{-3}. The ASAT value of *Oncorhynchus mykiss* is 38.54±8.88 (Jafarpour & Nekuie 2016).

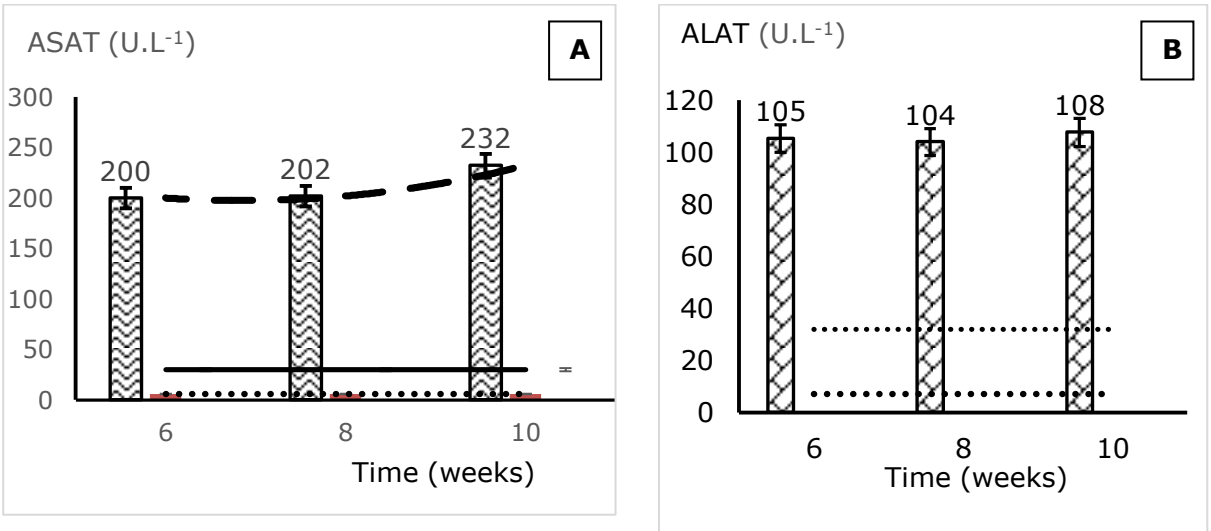

Figure 2. Aminotransferase enzymes in *Clarias gariepinus* reared with biofloc technology.

The ALAT enzymes concentration of *C. gariepinus* cultivated with stocking density of 1,000 individuals m^{-3} and applications of BFT is high, i.e. 104 to 108 U/L (Figure 2b). The value is above normal (<41 U L^{-1}) (Pratt 2010). The value of ALAT enzyme is higher than the value of ALAT enzyme in *C. gariepinus* cultivated using density 200 individuals m^{-3} and water flow, which is 63.67±3.20 U.L^{-1} (Hastuti & Subandiyono 2013). The value of

ALAT enzyme in salmon (*O. mykiss*) was 44.51±6.09 U L^{-1} (Jafarpour & Nekuie 2016). The value of ALAT enzyme of *C. gariepinus* is high (Hastuti & Subandiyono 2013), but the value increases with increasing stocking density. *C. gariepinus* cultivated in high stocking densities leads to increase in the value of ALAT in blood.

Blood parameters evaluation can be a method to determine the stress response of fish (Atmadi et al 2016; Barton et al 2002). Response of fish stress is an available adaptation mechanism of fish to overcome the stressor in order to keep itself in normal physiological condition (homeostatic state). Environmental stress will affect metabolism and growth, disease resistance, reproductive capacity, health conditions, and fish survival (Barton et al 2002).

C. gariepinus cultivated with high density, i.e. 1,000 individuals m^{-3} and using BFT system show stress condition, and therefore the fish have high blood glucose level (Figure 1d). Fish density conditions responded with rised direct bilirubin and blood glucose and decreased red blood cells (RBC). The RBC value of *C. gariepinus* cultivated with stocking density of 1,000 individuals m^{-3} and using BFT system is 1.6 to 1.7 million cells μL^{-1} (Figure 3a). The RBC value is lower than the value of RBC of *C. gariepinus* cultivated using a density of 200 individuals m^{-3} in running water, which was 2.30±0.06 million cells μL^{-1} (Hastuti & Subandiyono 2013). The low RBC value is caused by fish density and BFT application without change of water, so *C. gariepinus* suffer stress and increase RBC breakdown (RBC experiencing lysis). Under these conditions, hemoglobin (Hb) also has a faster breakdown so that the Hb value of fish is also low, at 6.4 to 7.5 g dL^{-1} (Figure 3c). The increase in Hb solving related to high value of direct billirubin (Figure 1b).

White blood cells (WBC) are a fish's defense system to ward off bacteria, viruses, germs, and other impurities that trigger a disease. The WBC defends body from disease by annihilating the pathogens through phagocytosis. Once the body detects an infection the bone marrow will produce more white blood cells to fight the infection.

WBC value of *C. gariepinus* cultivated with a stocking density of 1000 individuals m^{-3} and BFT application is 175,000 to 206,300 cells μL^{-1} (Figure 3b). WBC values are initially normal, but at the 10^{th} week decreases. Up to the 8^{th} week WBC values are still in normal levels, which means that *C. gariepinus* is in healthy condition. At the week 10, the WBC value decreased to 175,000 cells μL^{-1}. Decreasing of the WBC value indicates that the immune system of the fish is weak, so its body will be vulnerable to the threat of infections. Some disorders causes decreasing of WBC values which can lead to liver and spleen diseases, which can cause hypersplenism so the blood cells will be destroyed in a high rate.

The spleen serves to filter blood, which means abnormal cells, including red blood cells, white blood cells and old platelets, are retained and are damaged by the reticuloendothelial system (Adrika 2013). There are several causes and one of them is infection, therefore the spleen is not working properly. When the spleen over-filters the cellular elements in the blood it is called hypersplenism.

Blood profiles can be used to evaluate physiological responses of fish. Stress responses in animals can be seen from the changes of hormone levels of cortisol, blood glucose, hemoglobin, and hematocrits. Under stress conditions will decrease the number of red blood cells, hematocrit values and hemoglobin levels, while number of white blood cell count tends to increase. The results of this study indicate that WBC shows the opposite response, i.e. the WBC value tends to decrease in the week 10 (Figure 3b). While the values of RBC, Hb, hematocrit and platelets are in the low range (Figure 3).

The platelets value of *C. gariepinus* cultivated with a stocking density of 1,000 individuals m^{-3} and BFT applications is 1,100 to 9,300 cells μL^{-1}. The value is below the normal value (100,000 to 300,000 cells μL^{-1}). Platelets are cells that are involved in the process of hemostasis, and have a function in blood clotting. The main regulator of thrombocyte production is the thrombopoietin (TPO) hormone, which is primarily synthesized in the liver. If the liver cells of the fish are damaged then the synthesis of TPO can be disrupted and then leads to low platelet value.

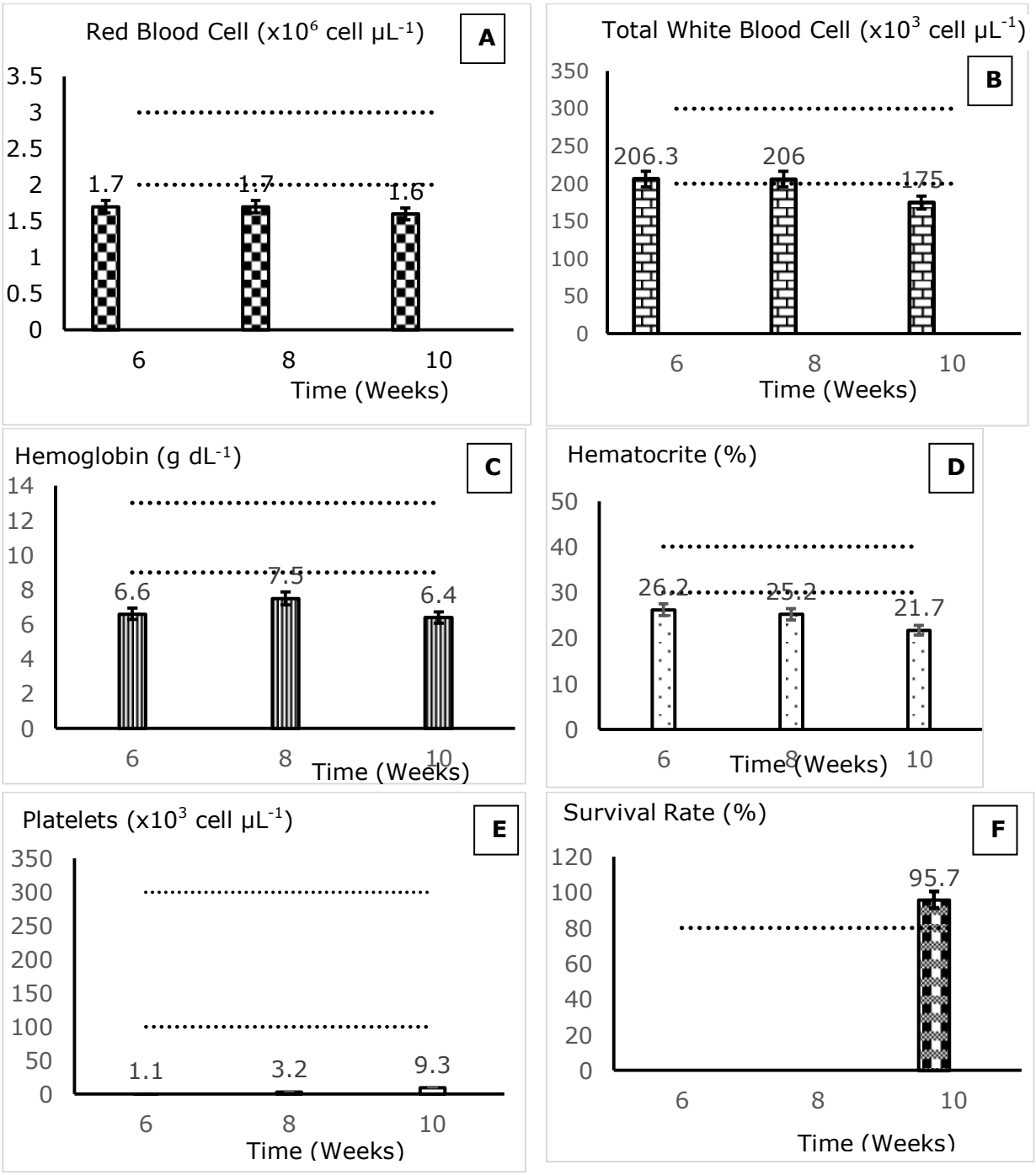

Figure 3. Haematological parameters and the survival rate of *Clarias gariepinus* cultivated with biofloc technology.

C. gariepinus is classified as a fish resistant to various stress conditions. In the density of 1,000 individuals m^{-3} and application of BFT, these fish show symptoms of low level various blood cells and high blood glucose. However, the fish is still able to survive with a survival rate value (SR) of 95.7±3.3% (Figure 3f). The SR value is higher than the SR value of *C. gariepinus* cultivated with running water, i.e. 81.16±2.0% (Hastuti & Subandiyono 2013). The high value of *C. gariepinus* SR is caused by application of BFT. The BFT system looks to be capable to support an adequate water environment required by *C. gariepinus*.

Water quality conditions during *C. gariepinus* cultivation for ten weeks using biofloc technology are presented in Figure 4. The value of turbidity during the *C. gariepinus* cultivation using biofloc technology has increased. The value of water turbidity

is 48 to 999 TU. The increase in turbidity means that during cultivation of fish, the water conditions become more turbid. The same condition was found in conductivity values. The conductivity value during cultivation was 3.7 to 497 S cm^{-1}. In contrast, pH values tend to decrease with a range of values of 8.21 to 7.55 (Figure 4c). At the beginning of cultivation, the dissolved oxygen value was 8.61 mg L^{-1}, subsequently decreased (Figure 4 d). The dissolved oxygen value oscillated in the range of 1.19 to 3.18 mg L^{-1}. Temperature values ranged from 30.4 to 27.9°C. Temperature values tend to be stable during the cultivation period with biofloc technology (Figure 4e). Figure 4f shows the total value of ammonia nitrogen (TAN) at the beginning of the cultivation which was 0 mg L^{-1}, furthermore, during the first two weeks the TAN value increased to 3.23 mg L^{-1}, and was persisted at a value of 0.26 to 1.36 mg L^{-1}. The increase of TAN value in the first two weeks is suspected because of the N input from fish while bacteria in biofloc are in a development phase, after which the growth of floc bacteria in the stationary phase so that the TAN value can be maintained in low concentration. The highest TAN value was 3.23 mg L^{-1}. Biofloc technology can improve water quality, especially TAN with no water exchange.

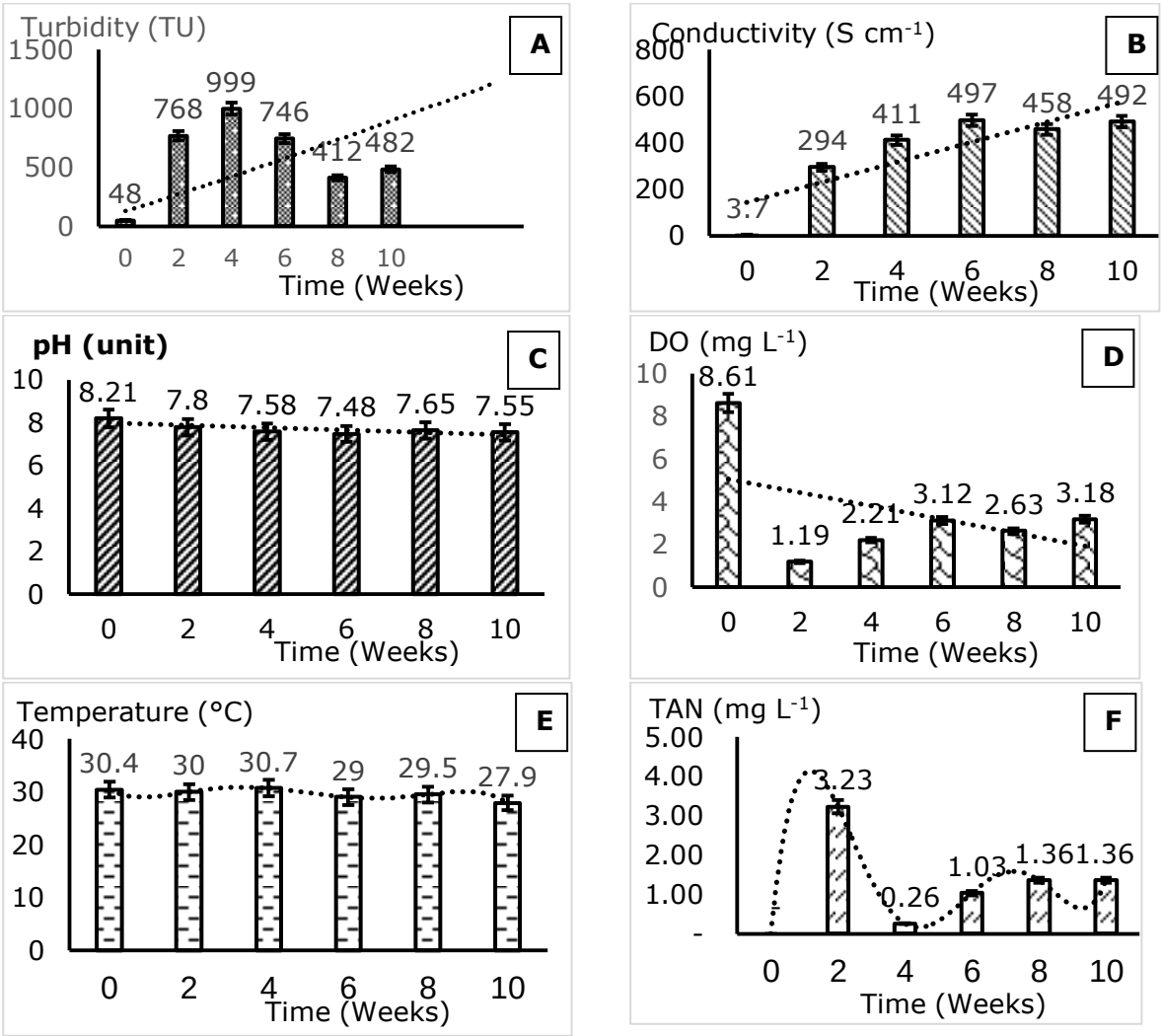

Figure 4. Water quality conditions during cultivation of *Clarias gariepinus* using biofloc technology.

Conclusions. The results of the present study concluded that cultivation of *C. gariepinus* with a density of 1,000 individuals m^{-3} applying biofloc technology shows low value of the haematological parameters concerning RBC, WBC, Hb, platelets and hematocrits. Total bilirubin and indirect bilirubin values were in normal ranges, while of direct bilirubin value was high. The enzyme ASAT, ALAT and blood glucose were in high concentrations. *C.*

gariepinus was in stress condition, and the recorded SR was 95.7±3.3%. Biofloc technology is able to maintain water quality in good conditions according to the *C. gariepinus* requirements.

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