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Abstract. The aim of this research was to study blood performances of jaundice and healthy catfish *Clarias gariepinus* (Burchell, 1822). Blood samples were collected from fifty fish (25 jaundice and 25 healthy catfish) and blood parameters were analyzed. The blood samples were collected from the caudal vein of the fish by using 2.5 mL syringe, and then collected into two recipients, one recipient with EDTA 1% anticoagulant and the other without anticoagulant. Blood samples in recipients without anticoagulant were used for blood cell counting and blood glucose analysis. Total bilirubin, direct and indirect bilirubin, and transaminase enzymes were determined from the blood serum samples with anticoagulant. Results showed that blood cell levels (leucocytes, erythrocytes, hemoglobin, and hematocrit) of jaundice catfish were lower compared to healthy catfish. The blood glucose concentration of jaundice catfish was higher compared to healthy catfish. The direct and indirect bilirubin concentrations of jaundice fish were also higher, which indicates that jaundice catfish had hyperbilirubinemia. Both jaundice and healthy catfish presented high concentrations of transaminase enzymes in the blood serum. Jaundice fish with hyperbilirubinemia presented liver malfunction and stress symptoms.

Key Words: African catfish, bilirubin, blood glucose, hematology, transaminase enzymes.

Introduction. African catfish *Clarias gariepinus* (Burchell, 1822) is a fast-growing fish (Muchlisin et al 2014), a rich source of animal protein, widely considered to be one of the most important tropical catfish species for aquaculture (Azua & Akaahan 2017; Kori-Siakpere et al 2011). The fish is omnivorous, feeding on fish, small mammals, invertebrates and also seeds and fruit. It lives in almost any freshwater habitat, but favours floodplains, large sluggish rivers, lakes and dams (Skelton 2001). In Indonesia, the regional name of the fish is "dumbo catfish". The fish has high economic value and is consumed by a large part of the Indonesian population. It was introduced in Indonesia in the early '80s (Hastuti & Subandiyono 2011; Sunarma et al 2017), and soon spread throughout the country, as it easily adapted to the tropical environment of Indonesia. Currently, dumbo catfish is widely farmed by the people of Indonesia. One of the nationally known catfish production regions is Kampung Lele village. Kampung Lele is located in Sawit, Boyolali district, Central Java.

Dumbo catfish has an additional breathing apparatus, namely the arborescent apparatus (Vivien et al 1977). Thus, it can be farmed in waters with relatively low dissolved oxygen (DO) levels. Moreover, the fish can be farmed in very high stocking density levels without experiencing oxygen deficiency issues. Super intensive systems are the aquaculture systems preferred in Kampung Lele village, Boyolali. Catfish farming implies high densities stocking (i.e. 300-500 fish/m^2) and utilizing commercial feeds. Unfortunately, the water management is very poor, resulting in diseases, one of which is jaundice. Jaundice disease appears in catfish farms near the harvest period. It is very harmful, and the price of infected catfish can drop to 20-40% of the healthy fish price.

Liver is a very important organ of fish and it has a function in maintaining the fish health (Holt & Smith 2008; Wang et al 2016). Hepatocytes comprise about 60% of total cells and 80% of the liver volume fulfills the metabolic and detoxifying needs of the body (Malarkey et al 2005; Gao et al 2007; Bogdanos et al 2013). These cells play pivotal roles in metabolism, detoxification and protein synthesis (Zhou et al 2016). The liver is located in the cranial region of the abdominal cavity and it is situated cranially to the stomach and foregut, ventral to the swimbladder. It plays a role in detoxification, production of vitollogenin as well as the deposition and metabolism of carbohydrates and fat (Sales et al 2017; Puslé and Anadon 1996). Vaghbijiani (2008) stated that liver acts as a regulator of amino acid levels in the blood by forming building blocks of protein. The liver also acts in converting toxic ammonia into urea; synthesizing and metabolizing cholesterol, phospholipids, triglycerides and lipoproteins; producing bile, which helps to carry the rest of the fat breakdown in the small intestine during the digestive process. Fat and fat soluble vitamins (vitamin A, D, E, and K) require bile for absorption. The liver functions in conjugating and excreting bilirubin. Other functions of the liver are inactivating hormones, metabolism and excreting drugs and toxins, defense of the body against infections through the production of immune factors and eliminating bacteria from the bloodstream. Major components of bile are bile salts, phospholipids, cholesterol, bilirubin, alkaline hydrogen carbonate ions (HCO_3^-) and water. The liver secretes bile via the bile ducts and the gallbladder into the intestinal lumen (duodenum). Bile produced by the hepatocytes is excreted into the bile canaliculi that empty into the bile ductule, which is formed by cholangiocytes (Hoeke 2013).

Jaundice in catfish is associated with yellow skin pigmentation. Yellow pigmentation is an indicator of increased bilirubin production or decreased bilirubin elimination by the liver (Clayton 2009). Bilirubin is the final product of haem destruction. Haem is largely derived from the breakdown of red blood cells or erythrocytes (80-85%), and a small part of the haem comes from a protein containing haem, cytochrome P450. The haem will undergo a number of complex chemical reactions to become bilirubin. The presence of bilirubin serum in higher levels indicates the increase of haem breakdown and the decrease of total bilirubin intake by the liver. Bilirubin conjugation takes place in the liver and, therefore, biliary excretion from the liver will decrease. These phenomena are related to the function of the liver. The condition of the liver functions is reflected by transaminase enzymes.

The aim of this study is to determine the blood performances of jaundice catfish, including blood cell levels, blood glucose, bilirubin and transaminase enzyme concentration in jaundice catfish compared to healthy catfish.

Material and Method

Blood sample preparation. In this study, samples of jaundice catfish and healthy catfish were taken from the same ponds to identify the hematological and enzymatic parameters. A total number of 50 fish, 25 jaundice and 25 healthy catfish were selected from farming ponds in Kampung Lele village, Boyolali district, Central Java. The jaundice catfish were identified based on skin coloration. The study was conducted from July to December 2017. Fish with the average body length and weight of 60±20 g and 24±5 cm, respectively, were collected**.** Blood samples were collected from the selected fish from the caudal vein, using a 2.5 mL-syringe. The blood samples were deposited in 2 types of containers, with 1% EDTA anticoagulant and without anticoagulant. Blood cell count and blood glucose analysis were performed for blood samples from containers with anticoagulant, while the analysis of bilirubin concentration and transaminase enzymes was carried out for blood samples from containers without anticoagulant.

Experimental design. This research used an explorative design, by selecting individuals of jaundice and healthy catfish in the field. Blood samples were collected from the selected fish. The blood samples were used for blood cell count, blood glucose, bilirubin, and transaminase enzymes. As supporting data, water quality parameters were determined. The data obtained was analyzed in Microsoft Excel and compared to normal values.

Variables and measurement methods. Leukocytes, erythrocytes, hemoglobin, hematocrit, and platelets were calculated photometrically, by using the ABX MICROS 60 tool. The device was connected to a printer, so measurements were printed out directly.

Blood glucose was analyzed by ON Call Plus. Blood glucose concentrations were determined based on the enzymatic reactions between glucose in blood samples with glucose oxidase to form gluconic acid, which then reacts with ferrocyanide to form ferrocyanide and producing potassium ferrocyanide. Potassium ferrocyanide was partially formed due to blood glucose levels. The oxidation of potassium ferrocyanide produces an electric current which is then converted into glucose concentration. Total bilirubin and direct bilirubin were measured by the photometric method, a modification of the Jendrassik and Grof method (Jendrassik & Grof 1938, Ngashangva et al 2019). The principle of the method is that bilirubin reacts with DSA (diazotized sulfanilic acid), producing a red color. The absorbance value of 546 nm wavelength is a directly related to bilirubin concentration. The water-soluble glucuronide bilirubin reacts directly with the DSA, whereas the indirect bilirubin conjugated with albumin will only respond to the DSA if there is an accelerator. Thus, total bilirubin equals the sum of direct and indirect bilirubin. The equation of the reaction is as follows:

Sulfanilic acid + sodium nitrite → DSA
Bilirubin + DSA → direct bilirubin
Bilirubin + DSA + accelerator → total azobilirubin (Jendrassik & Grof 1938)

Transaminase enzyme activity, which consists of GPT (glutamic-pyruvic transaminase) and GOT (glutamic oxaloacetic transaminase) in the blood serum of jaundice and healthy catfish, was determined by the kinetic method and read by UV photometric technology (Huang et al 2006; Wang et al 2016). The GPT or ALAT[a] (alanine aminotransferase) and GOT or ASAT[b] (aspartate aminotransferase) were measured by the reaction principles as follows:

$$\text{2-oxaloglutarate} + \text{L-alanine} \xrightleftharpoons{\text{GPT}} \text{L-glutamate} + \text{pyruvate}$$

$$\text{Pyruvate} + \text{NADH} + H^{+} \xrightleftharpoons{\text{LDH}} \text{L-lactate} + \text{NAD}^{+a}$$

$$\text{2-oxaloglutarat} + \text{L-aspartate} \xrightleftharpoons{\text{GOT}} \text{L-glutamate} + \text{oxaloacetate}$$

$$\text{Oxaloacetate} + \text{NADH} + H^{+} \xrightleftharpoons{\text{MDH}} \text{L-malate} + \text{NAD}^{+b}$$

Results. The results of this study, both for jaundice and healthy catfish, in super-intensive cultivation systems in Kampung Lele village are presented in Figure 1. The mean values of total leukocyte count of jaundice and healthy catfish were 73.8 (x10^3 cell μL^{-1}) and 126.1 (x10^3 cell μL^{-1}), respectively. The erythrocyte counts of jaundice and healthy catfish were 0.6 million cells μL^{-1} and 2.4 million cells μL^{-1}, respectively. The hemoglobin value in healthy catfish blood was 11.4 g dL^{-1}, while in jaundice catfish was 3.1 g dL^{-1}. The average hematocrit values in jaundice and healthy catfish were 8.2% and 32.7%, respectively.

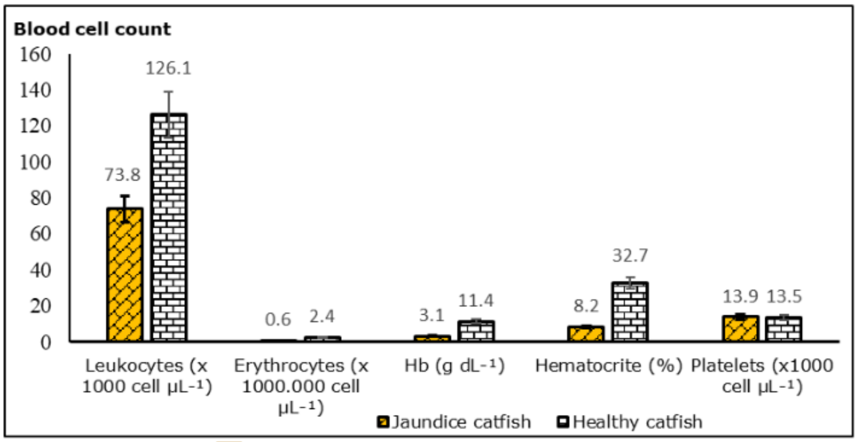

Figure 1. The number of blood cell counts of jaundice and healthy African catfish (*Clarias gariepinus*).

The results of bilirubin measurements, which consist of total bilirubin, direct bilirubin, and indirect bilirubin, are presented in Figure 2.

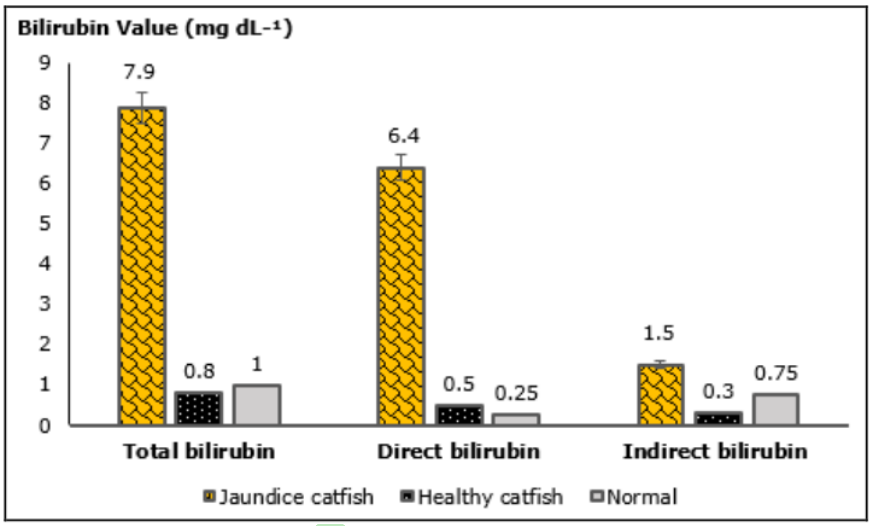

Figure 2. Bilirubin concentration in the blood of jaundice and healthy African catfish (*C. gariepinus*).

The values of transaminase enzymes in the blood of jaundice catfish were 372.6 UL^{-1} for SGPT (serum GPT) and 171.1 UL^{-1} for SGOT (serum GOT). The values of transaminase enzymes in healthy catfish blood were 124.2 UL^{-1} and 40.0 UL^{-1} for SGPT and SGOT, respectively (Figure 3).

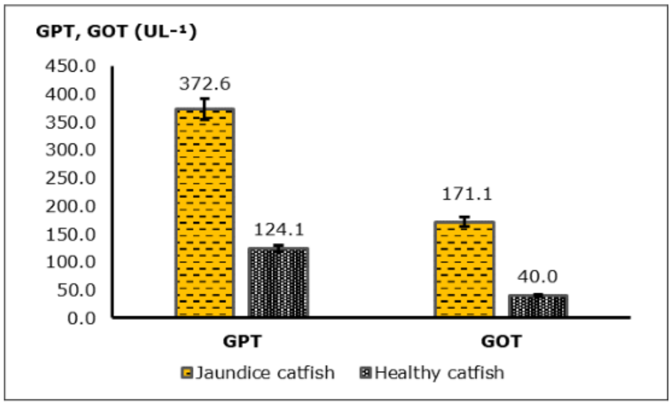

Figure 3. Transaminase enzyme (GPT and GOT) concentration in jaundice and healthy African catfish (*Clarias gariepinus*) serum.

Blood glucose concentration of jaundice catfish was higher compared to healthy catfish (Figure 4). The mean values of blood glucose concentration of jaundice and healthy catfish were 201.6 dL^{-1} and 50.6 mg dL^{-1}, respectively. Those values were obtained from population individuals that had been in fasting condition for four days.

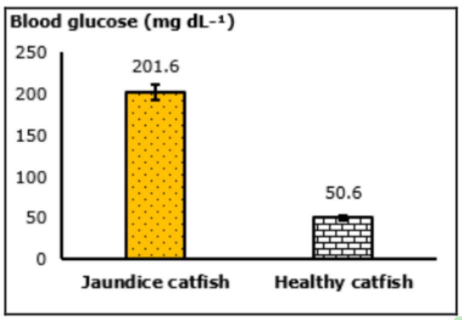

Figure 4. Blood glucose concentrations of jaundice and healthy African catfish (*Clarias gariepinus*).

Water quality is one of the most supporting factors of catfish life. The water quality in catfish farming ponds in Kampung Lele village, Boyolali district are presented in Table 1. The value of total ammonia nitrogen (TAN) and NH_3 markedly increased during the rearing period.

Table 1
The water quality parameters from African catfish (*Clarias gariepinus*) ponds

No.	Water quality parameters	Values
1	Total ammonia nitrogen (TAN) (mg L^{-1})	>400
2	Ammonia (NH_3) (mg L^{-1})	10.88±1.02
3	Temperature (°C)	28.9±1.03
4	Nitrite (NO_2) (mg L^{-1})	0.05±0.01
5	pH (unit)	7.51±0.31
6	Dissolved oxygen (mg L^{-1})	3.51±0.34
7	Water exchange	No exchange

Discussion. Jaundice represents a yellow coloration of the skin and other tissue due to an excess of bile pigment present in the blood and lymph. The affected fish suffer from haemolytic anaemia, resulting in jaundice (Chinabut 2002). Alteration of blood cell numbers, as well as biochemical and hormonal status might be an indication of unsuitable environmental conditions (Hastuti & Subandiyono 2016; Hastuti & Subandiyono 2018). The blood cells consisting of total leucocytes, erythrocytes, hemoglobin, and hematocrit in jaundice catfish were compared to healthy catfish (Figure 1). However, the two groups of catfish have no different platelet counts. Various blood cell levels of the jaundice catfish were lower than those of healthy catfish. Compared to blood cell numbers of normal values in African catfish, total cell count of leucocytes, erythrocytes, hemoglobin, and hematocrit of jaundice catfish were lower, except for the platelet count, which presented normal levels. Based on total leukocyte cells, jaundice catfish show lower health status than normal catfish.

In terms of direct bilirubin, the jaundice and healthy catfish have direct bilirubin values greater than the normal values. This phenomenon revealed that jaundice and healthy catfish underwent hemolysis or hemoglobin breakdown of their erythrocyte cells rapidly. However, in relation to hemoglobin values, jaundice catfish showed a faster hemolysis process (Figure 1). This statement is reinforced by the low hemoglobin value in the blood of jaundice catfish, which was 3.1 g dL^{-1} (Figure 1). A similar bio-physiological phenomenon was observed in jaundice catfish. Hematocrit values of the jaundice catfish were lower than in healthy catfish (Figure 1). The two groups of fish contained hematocrit values lower than the normal hematocrit value of catfish (37% to 50%). The hematocrit percentage of catfish reflects the proportion of erythrocytes in the blood, and may also describe the size of erythrocyte cells.

The hematological performances of fish describe the health condition (Jhonny et al 2003). Results show that the blood cell count of jaundice catfish characterize a poor health condition. The health condition was considered to be related to liver function damage (Abbas et al 2016). Cell damage or abnormalities in the liver caused the fish color to become yellow or hyper-bilirubin. Moreover, it caused the increase of transaminase enzymes consisting of SGPT and SGOT in the blood serum (Figure 3). Jaundice indicates hyperbilirubinemia, where bilirubin may be conjugated, which causes a yellowing of the skin (Abbas et al 2016). Blood serum of the jaundice catfish contains GPT-enzyme 11 to 12 times higher than normal value (Pratt 2010).

Jaundice catfish contained higher concentration of total bilirubin, direct bilirubin and indirect bilirubin compared to healthy catfish (Figure 2). The total concentration of bilirubin and direct bilirubin in jaundice catfish were 7 to 8 times higher than the normal bilirubin concentration, while indirect bilirubin concentrations were only 2 times higher than normal concentration. Bilirubin is an endogenously synthesized yellow-orange bile pigment that can be toxic if it is deposited. It causes a yellowing of skin, mucous membranes and sclera (Tiribelli & Ostrow 2005).

Jaundice is actually the high bilirubin level in the body. Bilirubin is produced by the breakdown of heme, a component derived from the hemoglobin of red blood cells or from other hemoproteins, such as myoglobin, cytochromes, and catalase. Heme oxygenase (HO), a rate-limiting enzyme in heme catabolism, cleaves heme to form biliverdin, which is subsequently converted by biliverdin reductase into bilirubin (Abbas et al 2016; Tsai & Tarng 2019).

Bilirubin is secreted through the bile and excreted through feces and urine. In the body, bilirubin is present in two forms, direct bilirubin bound to albumin and indirect bilirubin, namely bilirubin which was released in association with albumin and conjugated with glucoronic acid. Increased concentration of direct bilirubin in the blood represents an imbalance between the breakdown of haem to bilirubin and the ability of the liver to make conjugated bilirubin (Abbas et al 2016; Tsai & Tarng 2019). This condition was probably caused by liver function damage and excessive red blood cell destruction. The high concentration of indirect bilirubin in the blood indicated bile duct injury.

According to Vaghbijiani (2008), bilirubin metabolism starts from hemoglobin in the red blood cells (erythrocytes) that break down and produce bilirubin bound to albumin. Furthermore, the bilirubin will enter the liver and undergo a process of conjugation by replacing the albumin bond with glucoronic acid. Through the bile ducts, bilirubin will be secreted into the gastrointestinal tract. Bilirubin is excreted through feces and urine in the form of urobilinogen.

Healthy catfish presented a lower total bilirubin concentration than the normal value. The same can be said about indirect bilirubin concentration. The direct bilirubin concentration in the blood of healthy catfish increased beyond the normal value (Figure 2). The increase of direct bilirubin concentration in healthy catfish could have had several causes, like the increase of haem destruction in the erythrocytes and the impairment of liver cell function. Thus, direct bilirubin presents difficulties in undergoing a conjugation process in the liver.

Figure 2 shows that the jaundice and healthy catfish contained greater direct bilirubin concentrations than the normal direct bilirubin. Therefore, it was suspected that the two groups of catfish suffered liver damage. However, the jaundice catfish suffered liver damage in a higher level compared to the liver damage in healthy catfish. The jaundice catfish also presented damage in the bile duct (Figure 2). Liver malfunction can also be observed from the serum transaminase values (Figure 3).

Figure 3 showed that the jaundice and healthy catfish have blood transaminase enzyme values greater than the normal values. Blood serum of the jaundice and healthy catfish contains transaminase enzymes (GPT or ALAT and GOT or ASAT) with greater concentration than the normal values (Figure 3). Blood serum of the healthy catfish contained GPT-enzymes 3 to 4 times higher than the normal value. GOT-enzyme concentrations in the blood serum both jaundice and healthy catfish reached 5 to 6 times and 2 to 3 times higher than the normal value, respectively. The increased level of transaminase enzymes in the fish blood showed impaired liver function (Hughes 2008). Transaminases, both glutamate oxaloacetate transaminase (SGOT) and glutamate pyruvate transaminase (SGPT), are enzymes produced from liver cells (Hughes 2008; Wang et al 2016). If the liver cells are damaged, it will result in an increase of the level of enzymes in the blood. The normal values for SGPT and SGOT are 7 to 32 UL^{-1} and 6 to 30 UL^{-1}, respectively (Figure 3). The average value of GPT (or ALAT) in salmon was 44.51±6.09 UL^{-1} and the value of GOT (or ASAT) was 38.54±8.88 UL^{-1} (Jafarpour & Nekuie 2016).

Based on the SGPT and SGOT data, it can be concluded that the jaundice and healthy catfish farmed in super-intensive systems show liver function damage. The liver function damage of jaundice catfish seemed to be more serious than the damage of liver function in healthy catfish. Jaundice catfish also showed high values of total, direct, and indirect bilirubin. It suggests that jaundice catfish showed bile duct damage. The major cause of jaundice is extra-hepatic biliary obstruction which can be classified into two types: idiopathic dilation of common bile duct and bile duct obstruction (Abbas et al 2016).

African catfish is a freshwater fish which is resistant to high density stress conditions (Hastuti & Subandiyono 2018). However, jaundice catfish presented higher blood glucose values than healthy catfish. The increase in blood glucose concentration could indicate stress (Hastuti et al 2003). Stress influences liver performance. Continuous stress conditions cause difficulty for the normal activity of liver functions. This would lead to more severe liver dysfunctions, and resulting in hyperbilirubinemia in jaundice catfish.

In terms of farming systems in Kampung Lele village, Boyolali, where stocking densities of 300 to 500 fish m^{-2} were applied and with no water exchange (Table 1), it was suspected that these conditions acted as an indirect trigger for stress. The water quality conditions were very poor. The total ammonia nitrogen (TAN) level was very high, more than 400 mg L^{-1} (Table 1). Such water quality conditions also cause stress to the cultured fish (Hastuti et al 2003). Environmental stress might cause an increase in serum glucose concentration (Patriche et al 2011). Intensive fish farming systems, high stocking densities corroborated with insufficient water change in the rearing units could markedly decrease water quality, resulting in stress and fish growth and development deficiencies (De las Heras et al 2015; Oprea et al 2015). According to Subandiyono & Hastuti (2008a, b), the optimum stocking density for catfish is 200 fish m^2. Catfish densities of more than 200 fish m^2 result in lower average size for fish and decreased fish immunity.

Conclusions. Based on the study, the levels of various types of blood cells in jaundice African catfish (*C. gariepinus*) are lower compared to healthy catfish, except for the platelet cells. The platelet cell numbers of jaundice and healthy catfish blood are similar. The jaundice catfish show the bio-physiological condition of hyperbilirubinemia. Transaminase enzyme concentrations in blood serum of both jaundice and healthy catfish are significantly higher than the normal level. However, the enzyme concentration of jaundice catfish markedly increased during farming period, compared to healthy catfish. Blood glucose level during fasting state of jaundice catfish was much higher than healthy catfish levels. However, both jaundice and healthy catfish levels of glucose remain higher than the normal level. Both jaundice and healthy catfish underwent environmental stress, although catfish in both groups possessed different individual stress resistance. The jaundice catfish seemed to be more affected.

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