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Correlation Of Water Quality Factors and Stock Density to Glucose and Cortisol Levels in Cantang Grouper (*Epinephelus fuscoguttatus* X *Epinephelus lanceolatus*) Raised in Conventional Ponds

Hubungan Faktor Kualitas Air Dan Padat Tebar Ikan Terhadap Kadar Glukosa Dan Kortisol Pada Kerapu Cantang (*Epinephelus fuscoguttatus* X *Epinephelus lanceolatus*) Yang dipelihara pada Kolam Konvensional

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ABSTRACT

The purpose of this study was to find a correlation between density and water quality with glucose and cortisol levels in Cantang grouper (*Epinephelus fuscoguttatus x Epinephelus lanceolatus*) reared in ponds as an indicator of stress. This study uses a time series sampling method in its implementation. There were 10 Cantang grouper samples taken in this study per pond with sizes ranging from 20-30 cm. Fish samples were taken from 3 different ponds so that a total of 30 cantang grouper samples were found, while 1.5 L of water samples were taken from each pond. The results of measuring glucose levels showed that the highest average glucose levels in fish blood were obtained in pond number 6 (51.22 ± 14.9 mg dl^{-1}) while the lowest was in pool number 5 (40.95 ± 15.2 mg dl^{-1}). Similar to glucose levels, the results of blood cortisol measurements showed that the highest average cortisol levels in fish blood were obtained in pond number 6 (116.45 ± 5.49 ng ml^{-1}) while the lowest was in pool number 5 (112.64 ± 4.55 ng ml^{-1}). Based on the results of the regression analysis between the variables of water quality factors and stocking density on glucose levels, an R-value of 0.729 is obtained, which means that there is a 72.9% relationship between the variables of water quality factors and stocking density on glucose (moderate relationship). While the results of the regression analysis between the variables of water quality factors and stocking density on cortisol levels, an R-value of 0.722 was obtained, which means that there is a 72.2% relationship between the variables of water quality factors and stocking density on cortisol (moderate relationship). According to this research study, water quality factors that had a significant effect ($p>0.05$) on glucose levels in Cantang grouper were Temperature, DO, Salinity and Ammonia.

ABSTRAK

Tujuan penelitian ini adalah untuk mengetahui hubungan kepadatan dan kualitas air dengan kadar glukosa dan kortisol pada ikan kerapu Cantang (Epinephelus fuscoguttatus x Epinephelus lanceolatus) yang dipelihara di kolam sebagai indikator stres. Penelitian ini menggunakan metode time series sampling dalam pelaksanaannya. Sampel ikan kerapu Cantang yang diambil pada penelitian ini sebanyak 10 buah per kolam dengan ukuran berkisar antara 20-30 cm. Sampel ikan diambil dari 3 kolam yang berbeda sehingga diperoleh total 30 sampel ikan kerapu cantang, sedangkan sampel air diambil dari masing-masing kolam sebanyak 1,5 L. Hasil pengukuran kadar glukosa menunjukkan rata-rata kadar glukosa darah ikan tertinggi diperoleh pada kolam nomor 6 (51,22 ± 14,9 mg.dl^{-1}) sedangkan terendah pada kolam nomor 5 (40,95 ± 15,2 mg.dl^{-1}). Sama halnya dengan kadar glukosa, hasil pengukuran kortisol darah menunjukkan bahwa rata-rata kadar kortisol darah ikan tertinggi diperoleh pada kolam nomor 6 (116,45 ± 5,49 ng ml-1) sedangkan terendah pada kolam nomor 5 (112,64 ± 4,55 ng.ml^{-1}). Berdasarkan hasil analisis regresi antara variabel faktor kualitas air dan padat penebaran terhadap kadar glukosa, diperoleh nilai R sebesar 0,729 yang berarti terdapat hubungan sebesar 72,9% antara variabel kualitas air. faktor dan kepadatan persediaan glukosa (hubungan sedang). Sedangkan hasil analisis regresi antara variabel faktor kualitas air dan kepadatan penebaran terhadap kadar kortisol diperoleh nilai R sebesar 0,722 yang berarti terdapat hubungan sebesar 72,2% antara variabel faktor kualitas air. dan kepadatan persediaan pada kortisol (hubungan sedang). Berdasarkan penelitian ini, faktor kualitas air yang berpengaruh nyata (p>0,05) terhadap kadar glukosa ikan kerapu Cantang adalah Suhu, DO, Salinitas dan Amoniak.

Kata Kunci	*Kadar kortisol, padat tebar, kadar gula darah, indikator stres, kualitas air*
Keywords	*Cortosol level, Stock density, Glucose level, Stress indicator, Water quality*
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INTRODUCTION

Grouper fish (*Epinephelus* sp.) is one of Indonesia's fishery commodities which has great opportunities because of its high selling value. Cantang grouper (*Epinephelus fuscoguttatus* x *Epinephelus lanceolatus*) is a hybrid fish product between female Tiger grouper (*E. fuscoguttatus*) and male Kertang grouper (*E. lanceolatus*). Prices on the Indonesian market range from IDR 110,000-120,0000 per kilo (KKP, 2018). As cultivation technology has progressed, almost all

Grouper species have become important export commodities, especially for Hong Kong, Japan, Singapore and China (Dedi et al., 2018). The export value of grouper in Indonesia was recorded at US$ 16.42 million in 2017, but this export value still does not meet the demand for the export market for grouper every year which continues to increase by

30.75% per year (KKP, 2018). So it is necessary to increase the acceleration of grouper aquaculture production, especially the Cantang grouper species (*Epinephelus fuscoguttatus* x *Epinephelus lanceolatus*) so that they can take the opportunity to meet the demand for the export market which continues to increase every year.

The successful cultivation of Cantang grouper is influenced by internal and external factors. Internal factors include the physiological characteristics of fish, fish size, and fish quality (health), while external factors include water quality (temperature, DO, pH, CO_2 and ammonia) and stocking density of fish in aquaculture ponds. The process of fish farming activities always causes competition in terms of the use of space, oxygen and food in the rearing medium by the commodities we are cultivating (Djauhari et al., 2019). Water quality that is not optimal and high stocking densities have a high chance of causing stress to fish and even death (Schulte, 2014). Stress is several physiological responses from the body that occurs when animals try to maintain a homeostatic state in their bodies (Taqwa, 2008). One of the causes of stress that occurs in fish is poor environmental conditions (Susanto et al., 2014). Under stressful conditions, fish experience a primary response and a secondary response. The primary response is a change in state by the Central Nervous System (CNS) and the release of stress hormones, namely cortisol and catecholamines (adrenaline and epinephrine) into the bloodstream via the endocrine system. While the secondary response occurs as a result of the release of stress hormones which cause changes in blood and tissue chemistry such as increased blood glucose levels in fish (Taqwa, 2008).

Water quality factors that can cause a stress response in fish include temperature fluctuations, lack of oxygen and high organic matter content. Sudden temperature changes can increase stress events in fish and not infrequently also cause death in fish (Kordi, 2000). According to Hastuti et al. (2003), in poikilothermic animals, temperature changes will affect the high supply of blood glucose for thermogenesis. Dissolved oxygen is needed by all living things for respiration and metabolic processes. In addition, dissolved oxygen is also needed for the process of oxidation and reduction of organic matter by microorganisms (Salmin, 2005). Low oxygen levels can affect the slow growth rate of fish (Mahasri, 2006). According to Astuti et al. (2014), high organic matter content caused by leftover feed and metabolic waste can result in a decrease in oxygen and cause hypoxia in cultivated fish. This condition can cause stress and even death in aquatic biota.

Stocking density in the cultivation process that is too high can also affect the metabolic processes of fish. As long as the stress response occurs in fish, it will cause insulin inactivation which should be used to control glucose levels in the fish's blood. Besides that, the increase in stocking density for cultivation can also affect the availability of oxygen in the waters. The level of oxygen consumption is strongly influenced by the density factor because the dissolved oxygen content will decrease as the density increases. This high stocking density can also cause a decrease in water quality during cultivation if it is not controlled (Nasichah et al., 2016). Based on this background, this study aims to determine the correlation of water quality and density factors on glucose levels and cortisol levels in

Cantang grouper (*Epinephelus fuscoguttatus* x *Epinephelus lanceolatus*) cultured in ponds.

MATERIALS AND METHODS

A sampling of fish and pond water

A sampling of cantang grouper was carried out in ponds located in Cumpleng Hamlet, Brengkok Village, Brondong District, Lamongan, East Java. As many as 10 Cantang grouper fish were taken from each pond using nets. Fish size ranges from 15-30 cm. Samples were taken from ponds number 4, 5 and 6, each measuring 1,320 m^2, 2,234 m^2 and 9,278 m^2. Water samples were taken from the inlet and outlet points of ponds number 4, 5 and 6 using a 1.5-litre sample bottle and then put into a coolbox filled with ice gel.

Measurement of glucose levels

Measurement of glucose levels was carried out by taking blood using a 1 ml syringe filled with 10% EDTA which functions as an anticoagulant. Blood collection was carried out in the caudal vein, which is a blood vessel that is located right on the ventral part of the vertebrae (backbone) (Hidayaturrahmah, 2015). The blood obtained is then dripped onto the tip of the glucose strip which has been inserted into the One Touch Ultra Plus glucose test device. The results of glucose levels will be seen on the screen of the glucose test in units of mg/dL.

Measurement of cortisol levels

Cortisol levels were measured at the Microbiology Laboratory, Faculty of Fisheries and Maritime Affairs, Universitas Airlangga. The blood sample that has been obtained will be centrifuged at 3000 rpm for 15 minutes, and the serum that has been obtained will be used as a sample to measure cortisol levels in Cantang grouper, cortisol levels in Cantang groupers are measured using an ELISA tool Cortisol can be measured with serum obtained from samples Cantang grouper blood and analyzed using ELISA. The Cortisol Elisa kit consists of a standard solution of 0.5 ml, Standard Diluent of 3 ml, Streptavidin-HRP of 6 ml, Stop solution of 6 ml, Substrate solution A of 6 ml, Substrate solution B of 6 ml and Wash buffer of 20 ml. The steps taken were to prepare all reagents, samples and diluent standards, add samples and ELISA reagents to each incubation vessel for 1 hour at 37^{o}C, and then wash the plate 5 times. Add substrate solutions A and B, incubate for 10 minutes at 37oC, and finally add the stop solution and wait for the colour change, wait for 10 minutes, then put it into the Enzyme-Linked Immunosorbent Assay (ELISA) and the results will show the cortisol levels of the Cantang grouper (Sink et al., 2008).

Statistical model and analysis procedure

The data obtained were processed using multiple linear regression tests. The equation of the multiple linear regression model is Y = a + bX1 + bX2 + bXa. Regression analysis is used to study the effect of independent variables on dependent variables (Kadir,

2015). The data is processed using the SPSS application program which will then be explained descriptively to make it easier to understand (Kusriningrum, 2010). The value indicating the relationship between the water quality factor/density and fish glucose/cortisol levels can be determined using the correlation coefficient (R), then to determine the effect of the water quality factor/density on glucose/cortisol levels, the determination coefficient (R2) can be used. The analysis uses a confidence level of 95% (α = 0.05) (Kadir, 2015).

RESULTS

The results of measuring blood glucose and cortisol levels showed that the highest average glucose levels in fish blood were obtained in pond number 6 (51.22 ± 14.9 mg dl^{-1}) while the lowest average glucose level was in pool number 5 (40.95 ± 15.2 mg dl^{-1}). Similar to glucose levels, the results of blood cortisol measurements showed that the highest average glucose levels in fish blood were obtained in pond number 6 (116.45 ± 5.49 ng ml^{-1}) while the lowest average glucose levels were in pool number 5 (112.64 ± 4.55 ng ml^{-1}). This is thought to occur because all ponds have higher Salinity, Nitrate, Ammonia and BOD values than the maximum standards that should be for grouper aquaculture in ponds (Table 1). Especially at very high nitrate levels, reaching 1.20-1.43 ppm with a maximum standard that should be 0.06 ppm (Suharyadi, 2011). Ammonia is also very high, reaching 0.6-1.7 ppm with a maximum standard that should be 0.3 ppm (Firdaus et al., 2011). So that the unsuitable water quality can cause stress for the fish with a marked increase in glucose and cortisol levels in the blood of the Cantang grouper.

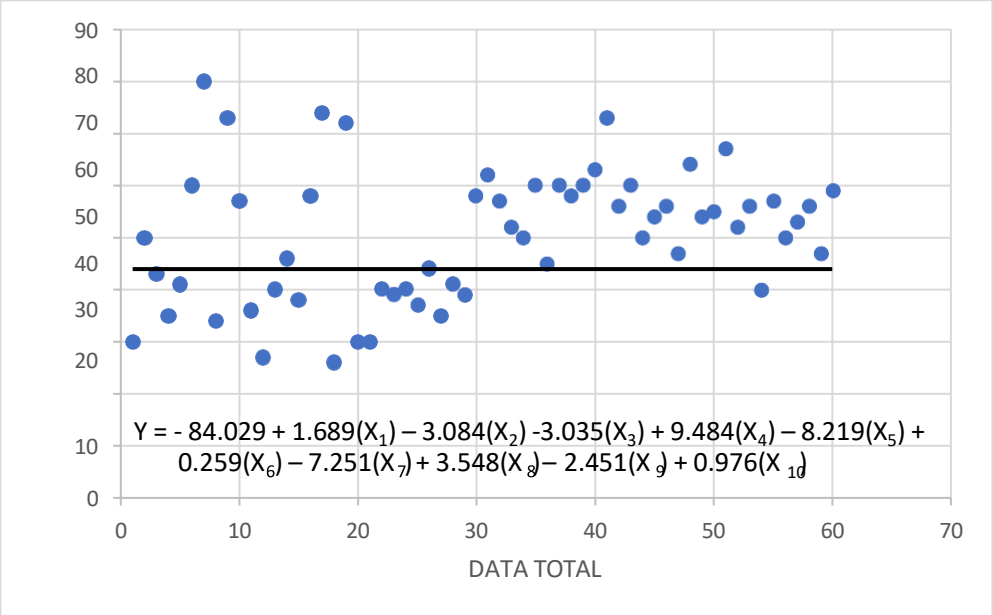

Figure 1. Linear regression graph of glucose on water quality factors and density

The results of measurements of length and weight showed that the average maximum weight was obtained in pond number 5 and the lowest weight was obtained in pond number 6. This is thought to be caused because pond 6 has the lowest growth rate due to high levels of glucose and cortisol in the pond in response to stress, so fish tend to use their energy to maintain metabolic balance in their bodies and energy is not used to increase their weight growth.

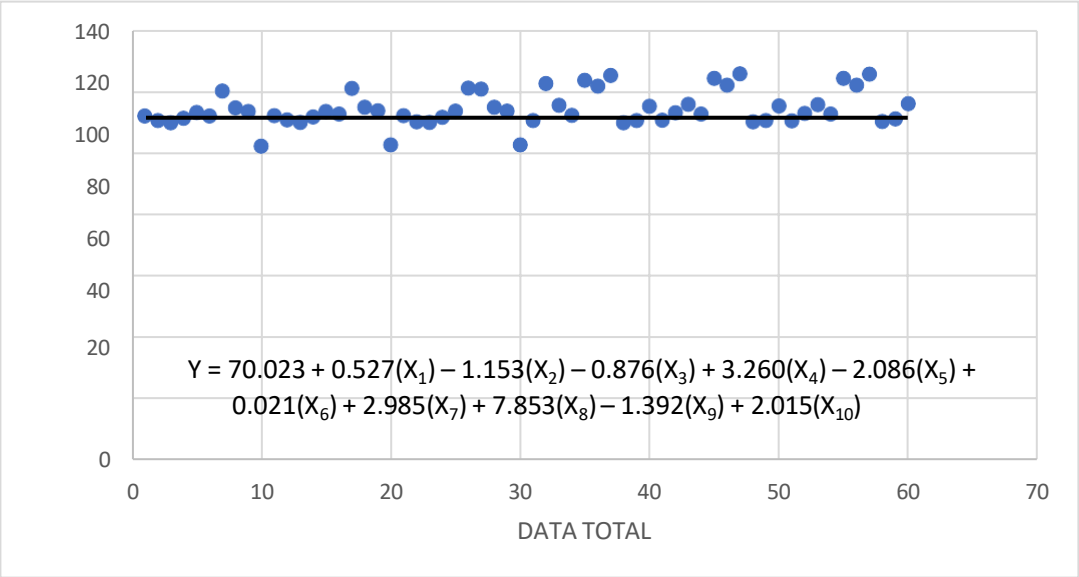

Figure 2. Linear regression graph of cortisol levels on water quality factors and density

Based on the results of the regression analysis between the variables of stocking density and water quality on glucose levels (Figure 1), an R-value of 0.729 is obtained, which means that there is a relationship of 72.9% (moderate relationship) between the variables of stocking density and water quality on glucose levels in Cantang grouper. On the results of the value of R Square, the results of the regression analysis showed a value of 0.532 which means that the Variable Stocking Density and Water Quality were able to influence the glucose levels of the Cantang grouper in the pond by 53.2%. With a significance level of 0.000 which indicates that Stocking Density and Water Quality had a significant effect ($P<0.05$) on the glucose levels of Cantang grouper.

Based on the results of the regression analysis between the variables of stocking density and water quality on cortisol levels (Figure 2), an R-value of 0.722 is obtained, which means that there is a 72.2% relationship between the variables of stocking density and water quality on cortisol (moderate relationship). Whereas the R Square value shows a value of 0.521 meaning that the Variable Stocking Density and Water Quality can affect Cortisol levels by 52.1%. With a significance level of 0.010 which indicated that stocking density and quality had a significant effect ($P<0.05$) on cortisol levels in Cantang grouper.

DISCUSSION

The results of water quality measurements in pond number 4 showed high levels of salinity, nitrate, ammonia, and BOD. This high level of salinity is possible because when the evaporation process occurs it causes the salt level in the waters to increase. Sudden and extreme fluctuations in salinity can be a stressor for fish and affect blood glucose and cortisol levels. It turns out that changes in salinity slowly still cause osmotic pressure if they are not following the tolerance threshold for fish (Evans & Kultz, 2020).

The results of water quality measurements in pond number 5 have better water quality compared to the other two ponds. Although the ammonia level in pond number 5 is still above the standard for grouper cultivation (Firdaus et al., 2011) it has the smallest level of 0.6 ppm when compared to ponds number 4 (1.7 ppm) and number 6 (1, 6 ppm). Pond number 5 has a slightly different cultivation system than the other two ponds because there is a separate paddle wheel and inlet and outlet. Paddle wheels are very influential in ponds because they can break down the density of water and allow dissolved oxygen levels to diffuse more quickly in the waters, besides that they also affect regulating the position of organic matter deposits or leftover feed and faeces in ponds (Suhendar et al., 2020). Apart from that, pond number 5 also has a water temperature 1 level above the standard for grouper cultivation, which is 32.1^{o}C (Affan, 2011). The results of the linear regression analysis in pond 5 showed that only temperature had a significant effect on the glucose levels of the Cantang grouper ($p<0.05$).

The results of water quality measurements in pond number 6 showed high levels of temperature, nitrate, nitrate, ammonia, and BOD. Increasing temperature can affect physiological processes in fish, such as metabolism, growth and reproduction. If the temperature increases, the solubility of oxygen will decrease, causing hypoxia in fish. This hypoxia then provides a secondary stress response in fish and causes glucose levels to increase. Increased glucose levels in fish exposed to high temperatures are caused by the mobilization of glycogen into glucose to meet the increased energy demands due to stress (Islam et al., 2019). Suhendar et al. (2020) suggested that high levels of ammonia in waters could be caused by the buildup of uneaten feed and the accumulation of faeces and the rest of the fish's body metabolism as the rearing time increases. Shokr (2015) in his research reported that there was an increase in glucose levels and cortisol levels in fish exposed to high ammonia in the waters. The increase in glucose levels most likely resulted from the glycogenolytic activity of catecholamines and gluconeogenetic glucocorticoids by the stress response due to exposure to toxic substances in the culture medium. The internal axis of the hypothalamus is stimulated by ammonia as a stressor so it will also increase cortisol levels in the fish's blood. After that, the processes of lipolysis, glycogenolysis and gluconeogenesis that occur will cause an increase in glucose levels to provide energy in stressful conditions. The results of multiple linear regression analysis showed that ammonia had a significant

effect on the glucose levels of the Cantang grouper ($p<0.05$).

The haematological approach is often used to detect physiological changes due to environmental stress and can also describe the health status of fish. Stress levels can generally be identified by measuring cortisol and glucose levels in the blood (Samsisko et al., 2014). Based on the results of testing glucose levels in Cantang grouper in ponds, it was stated that pond number 6 had the highest glucose level, namely 51.22 ± 14.9 mg dl^{-1}. Whereas ponds number 4 and number 5 respectively were 48.07 ± 11.3 mg dl^{-1} and 40.95 ± 15.2 mg dl^{-1}. According to Porchase et al. (2009) in their research stated that normal grouper glucose levels ranged from 28.8 to 34.2 mg dl^{-1}, so the results of sampling on the three ponds stated that the Cantang grouper sample was all experiencing stress when viewed from blood glucose levels.

The results of testing cortisol levels in Cantang grouper in ponds stated that pond number 6 had the highest cortisol levels, namely 116.45 ± 5.49 ng ml^{-1}. Whereas ponds number 4 and number 5 respectively were 114.50 ± 4.23 ng ml^{-1} and 112.64 ± 4.55 ng ml^{-1}. Van der Vyver et al. (2013) in his study stated that normal grouper cortisol levels ranged from ≥ 29 ng ml^{-1}. So the results of the sampling on the three ponds stated that all of the Cantang grouper samples were in a state of stress when viewed from cortisol levels in the blood. These stress conditions can be caused by external factors, especially the water quality factor which is above the standard threshold for grouper fish farming (Table 1) so it becomes the main trigger for increased glucose and cortisol levels.

This statement is supported by Lidhia et al. (2014) who state that one indication of fish experiencing stress is an increase in glucose levels in blood plasma. The existence of a stress response will stimulate the hypothalamus to release corticotrophin-releasing factor (CRF), and this CRF will stimulate the anterior pituitary gland to release the hormone Adrenocorticotropin Hormone (ACTH). ACTH then stimulates the interrenal cells (adrenal medulla) to produce Cortisol and Catecholamine hormones (eg, epinephrine). This hormone plays a role in the process of gluconeogenesis which will deposit glycogen reserves in the liver and muscles to increase glucose levels in the blood.

The stocking density of Cantang grouper culture at the sampling site (Brondong District, Lamongan City, East Java) has a stocking density of 14 individual m^{-2} (pond 4), 3 individual m^{-2} (pond 5), and 13 individual m^{-2} (pond 6) with grouper weight ranging from 100-400 g. The Ministry of Marine and Fisheries Ministry (2020) in the guidelines for good grouper cultivation states that the maximum stocking density for grouper cultivation with a length of 20-30 cm is 25- 30 individual m^{-2}. Septiawati (2010) in her research also revealed a similarity related to the stocking density of grouper, namely that in grouper rearing ponds at BBPBAP Jepara with a stocking density of 15 individuals m^{-2} at a size of 100-300 g. The results showed that the stocking density of Cantang grouper reared in ponds had a significant effect on blood glucose and cortisol levels of fish, based on the regression values of glucose levels and cortisol levels on stocking density values each had a significance value of 0.36

(36%) and 0.43 (46%). When compared with the regression values of DO (glucose: sig. 0.09; cortisol: sig. 0.13) and salinity (glucose: sig. 0.00; cortisol: 0.25) the density regression values are still greater, this indicates that stocking density has a smaller effect than DO and salinity in affecting glucose and cortisol levels of fish. According to Yudha & Kamal (2013) the smaller the significance value obtained, the greater the effect on the data. While the regression direction of stocking density is positive so that if the stocking density increases, the glucose and cortisol levels will increase and when the stocking density decreases, the glucose and cortisol levels will also decrease. We suspect that the small regression values of glucose levels and cortisol levels on stocking density are since the stocking density values in the three ponds are still under grouper culture standards so they do not exceed the maximum limit for density values, so the fish tend to get enough space to move and there is not too much competition in space motion (Folnuari et al., 2017).

CONCLUSIONS

There is a correlation between water quality and stocking density factors on glucose levels and cortisol levels as indicators of stress in Cantang grouper reared in traditional aquaculture ponds, Brondong District, Lamongan Regency, East Java, with a conversion value (R) of 0.729 for glucose and a conversion value (R) of 0.722 for cortisol levels. Water quality factors that had a significant effect ($p>0.05$) on glucose and cortisol levels in Cantang grouper were temperature, DO, salinity and ammonia. Stocking density also had a significant effect ($p>0.05$) on glucose levels and cortisol levels in the blood of Cantang grouper.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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