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Physiological and Hematological Responses in Cantang Grouper (*Epinephelus fuscoguttatus x Epinephelus lanceolatus*) Against the Salinity of Different Media

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Abstract

Fish Cantang grouper is a cross between a female tiger grouper (*Ephinepelus fuscoguttatus*) and a male kertang grouper (*E. lanceolatus*), has a high economic value. Salinity is a water quality factor that affects cultivation because salinity has osmotic pressure that can cause changes in physiological activity and stress in fish if it is not in accordance with the habitat. Stress in fish causes the release of cortisol and catecholamine hormones. The catecholamine hormone then increases the glycogenolysis process, regulates cardiovascular and respiratory function, in addition to increasing cortisol and blood glucose. Stress in fish is also indicated by a hematological response in the form of changes in the number of erythrocytes. This study aims to determine the effect of different salinity treatments on physiological and hematological responses. The results of the analysis of variance (ANOVA) carried out from the initial observation to 24 hours showed that each salinity treatment had a significantly different effect on blood cortisol, blood glucose, and erythrocyte levels, with a value ($p < 0.05$). The highest average blood cortisol was in the 36 ppt treatment at 24-hour observation time. The average blood glucose increased from before being treated until receiving treatment for up to 24 hours. The highest average was obtained at a salinity of 36 ppt, with observation for 24 hours. The results obtained from the study showed that the highest number of erythrocyte cells at 24 hours was in the P2 treatment (36 ppt), namely 1.15 x 10^6 cells/mm^3.

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INTRODUCTION

Indonesia is a vast maritime country, and there are many commodities from aquaculture, one of which is grouper (*Epinephelus* sp.). Grouper (*Epinephelus* sp.) is one of Indonesia's fishery commodities that has great potential for cultivation because its selling value is quite high. The demand for grouper fish increased from 6 tons in 2022 to 7 tons in 2023. One of the grouper fish commodities that is in great demand is the hybrid cantang grouper, which is the result of a cross between a female tiger grouper (*E. fuscoguttatus*) and a male kertang grouper (*E. lanceolatus*).

The cross between the two types of fish has the characteristic of being easy to breed, like tiger grouper, and has fast growth like tiger grouper (Triastuti *et al.*, 2017). This condition is thought to be because the hybridized cantang grouper inherited the growth gene from the kertang grouper, which is relatively high, also has a high appetite, and can utilize feed better to balance its metabolic rate (Sutarmat and Hirmawan, 2013). The high demand for grouper fish has caused the community to develop grouper fish seed businesses.

The high demand for grouper fish has caused people to develop grouper fish seeding businesses. The cultivation of cantang grouper fish itself must be carried out by paying attention to good and correct fish management techniques, starting from handling, limiting the number, cultivation techniques, water quality management, and transportation methods from seeding to the final stage. Water quality management is very important for the cultivation process to be successful; improper fish management can cause disturbances that can cause stress responses that lead to decreased fish performance, making them susceptible to disease and death in fish (Gabriel and Akinrotomi, 2011).

The development of grouper fish farming has various problems experienced by cantang grouper fish farmers, one of which is stress. During stressful conditions, fish experience increased cortisol and blood glucose (Martínez-Porchaz *et al.*, 2009). Stress affects cortisol levels, which can also be directly influenced by handling pressure during sampling, which is more stressful, detrimental, and problematic for fish than higher vertebrates, especially because they are removed from the water for a long time (Cao *et al.*, 2017). One of the stress response factors in the physiology of fish behavior from water chemistry that plays a role in survival is salinity. Salinity affects growth because salinity has osmotic pressure, which can cause changes in the physiological activity of fish; this occurs due to the higher concentration of seawater for fish (Muliani, 2016). Salinity is the concentration of ions dissolved in water. Salinity that is not within the habitat will cause stress in cantang grouper fish, which will have an impact on growth. Acute stress will have an impact on the physiological response of cantang grouper fish (Faozan *et al.*, 2019).

Stress caused by inappropriate salinity, such as in European bass, which secretes the highest mucus volume when exposed to hypersalinity with the highest total cortisol and glucose (Ordóñez *et al.*, 2020). Evan and Kültz (2020) in their study also stated that salinity stress can occur when the salt concentration in the environment changes rapidly, for example, due to tidal flow, storm rain, drought, or evaporation. Different salinity concentrations also put pressure on the physiology of exposed *Barbonymus gonionotus* fish (Amin *et al.*, 2016). Salinity stressors also have a significant effect on Hb, PCV, Na^{+}, K^{+}, Ca^{++}, and lactate in tilapia (Mohamed *et al.*, 2021).

Examination of physiological responses in fish due to stress can be done by taking blood and observing fish behavior. Parameters commonly used to diagnose fish stress levels are blood cortisol levels and blood glucose. Evaluation of hematological responses in fish due to stress can be done through blood tests. Parameters commonly used as an index to determine fish health status are total red blood cells. Under stressful conditions, there is a change in the

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number of erythrocytes in fish (Royan *et al.*, 2014). Therefore, this study was conducted to determine the physiological and hematological responses of cantang grouper (*E. fuscoguttatus x E. lanceolatus*) maintained in different media salinities.

METHODOLOGY

Ethical Approval

Cantang grouper fish obtained from BPBAP Situbondo, with a size of 7-9cm, as many as 336 fish per replication. Then acclimatized for 15 minutes, continued with the treatment of being maintained for 0; 0.5; 1; 2; 4; 8; and 24 hours, then the sampling process was anesthetized first by wrapping the fish's body using a cloth that had been soaked in cold water at a temperature of 15 °C. The anesthesia process was carried out for approximately one minute. Then, blood samples were taken without injuring the fish according to the procedure. Test animals were not treated improperly during the study.

Place and Time

The research activity was carried out from September 2023 to July 2024 using the experimental method. The research was carried out at the Anatomy and Cultivation Laboratory, Faculty of Fisheries and Marine Sciences, Airlangga University, Surabaya.

Research Materials

The tools that will be used in this study are aquariums measuring 45 cm x 45 cm x 60 cm, stacking racks, tank profiles, reservoirs, thermometers, aerators, hoses, aeration stones, DO meters, pH meters, refractometers, 1 ml syringes, hand counters, glucometer OneTouch, ELISA kit Cusabio (Cubio, Houston, USA) microtube, droppers, rags, scissors, tissues and newspapers.

The materials that will be used in this study are cantang grouper fish from BPBAP Situbondo with a length of 7-9 cm, seawater, freshwater, coarse salt, 10% EDTA, chlorine, distilled water, and ice cubes.

Research Design

The method used in this study is an experimental method by provides treatment. The research used in this study is a Completely Randomized Design (CRD) with 3 (three) treatments. Each treatment was repeated 6 (six) times. In the treatment study, as following: P0 (Control with maintenance at a salinity of 32 ppt), P1 (Cantang grouper fish are maintained at a salinity of 28 ppt), and P2 (Cantang grouper fish are maintained at a salinity of 36 ppt).

Work Procedure

Research Preparation

Preparation in this study began with the sterilization of tools and materials. The aquarium that will be used in this study was first cleaned by washing it with soap until clean to remove dirt from it. The maintenance container, aeration hose, and aeration stone that were cleaned were then soaked using chlorine for 24 hours. The water in the aquarium was then discarded, and the aquarium was washed again to remove any remaining chlorine. The aquarium can then be filled with 2 liters of seawater for a density of 1 fish measuring 7-9 cm with a total of 28 liters per 14 fish, then given aeration. Marking fish that have been used for treatment with fish that have not been used is done by putting them in another aquarium.

Maintenance

Fish are sent using plastic bags that are given additional oxygen. Before the fish are transferred to the maintenance container, the fish are acclimatized first in seawater media that has been provided with a suitable habitat salinity of 32 ppt for a day before treatment.

Blood Sampling

Blood sampling of cantang grouper fish was carried out at 0, 0.5, 1, 2, 4, 8, and 24 hours after the fish were put into the aquarium. The determination of the time for blood sampling refers to research conducted by Sulmartiwi *et al.* (2013). One fish used in

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the study had its blood drawn once and was first anesthetized with cold water before its blood was taken. The fish whose blood was to be taken were first anesthetized by wrapping the fish's body using a cloth that had been soaked in cold water at a temperature of 15 °C. The anesthesia process was carried out for approximately one minute. The blood sampling process was carried out through the caudalis vein using a 1 ml syringe that had previously been rinsed using EDTA solution as an anticoagulant. Blood samples were taken from the back of the anal fin towards the spine until the syringe needle touched the spine (Preanger *et al.*, 2016). The ratio of the EDTA solution needed to the fish blood taken was 1:9. The blood samples that had been taken were then put into a microtube and labeled according to the treatment and time of sampling (Zang *et al.*, 2013).

Blood Glucose

The blood that has been taken is then placed on the tip of the Easy Touch test script that has been activated with the Blood Glucose Monitoring System, and the blood glucose results are recorded.

Total Erythrocyte Count

The total erythrocyte count was done by sucking the blood sample of the cantang grouper using a Thoma erythrocyte pipette to a scale of 0.5, then adding Hayem's solution to a scale of 101. The blood sample was then homogenized in the pipette for 5-10 minutes. The first 3-4 drops of the blood sample in the pipette were discarded first, then the blood sample was dripped onto the Neubauer hemocytometer and covered with a cover glass. The counting chamber was then placed on the microscope, and the count was performed with a magnification of 400 times. The counting chamber used in erythrocyte counting is a small chamber located in the middle of the hemocytometer with 25 chambers. Of the 25 chambers, the four chambers in the corners plus one chamber in the middle were counted. The following is the formula for calculating the total number of erythrocytes according to Johnny *et al.* (2003).

$$\text{Total Erythrocyte} = \frac{A}{N} \text{x} \frac{1}{V} \text{xDf}$$

A : Number of erythrocyte cells counted
V : Volume of the haemocytometer box
N : Number of haemocytometer boxes observed
Df : Dilution factor

Measurement of Blood Cortisol Levels

Cortisol measurements were tested through serum obtained from 1 ml of cantang grouper blood sample, then tested using the Fish Cortisol Elisa Kit (Sulmartiwi *et al.*, 2022). The steps are as follows. Prepare reagents, samples and standards according to the instructions, then make a microplate without solution, then add 50 μl of standard or sample to each well, then add 50 μl of antibody (1x) to each well (not an empty well), incubate for 40 minutes at 37°C, aspirate and wash 3 times, then add 100 μl of HRP-conjugate (1x) to each well (not an empty well), incubate for 30 minutes at 37°C, aspirate and wash 5 times, add 90 μl of TMB Substrate to each well. Incubate for 20 minutes at 37°C. Protect from light 11. Add 50 milliliters of stop solution to each well. Read at 450 nm in 5 minutes. Calculation of results can use a curve from computer software that can produce a four-parameter logistic curve fit (4-PL).

Data Analysis

The research data were analyzed using the ANOVA test with SPSS 20 to show the significant effect ($p < 0.05$) of different salinity stressors on cortisol, blood glucose, and erythrocyte levels.

RESULTS AND DISCUSSIONS

Measurement of cortisol levels in the blood and mucus of cantang grouper fish was carried out at each hour, namely 0 hours, 0.5 hours, 1 hour, 2 hours, 4 hours, 8 hours, and 24 hours. Cortisol levels in the blood and mucus of cantang grouper fish vary in different sizes each hour. The results of the analysis of variance (ANOVA) carried out at the beginning of the observation until 24 hours showed that each salinity treatment

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had a significantly different effect on cortisol levels in the blood and mucus of cantang grouper fish (*E. fuscoguttatus* x *E. lanceolatus*) with salinity treatments of 28 ppt, 32 ppt, and 36 ppt as shown in Table 1.

Table 1. Average cortisol levels in blood (ng/mL) of cantang grouper *(E. fuscoguttatus* x *E. lanceolatus*) during the study ± SD.

Treatment Time (hours)	Salinity		
	32 ppt	28 ppt	36 ppt
0	35.91±2.16^{a}	48.66±10.08^{b}	57.57±4.46^{c}
0.5	59.10±7.80^{a}	52.60±20.30^{a}	88.86±10.83^{b}
1	68.13±3.74^{a}	69.63±29.63^{a}	102.97±13.06^{b}
2	97.40±17.25^{a}	81.21±4.10^{a}	133.49±28.02^{b}
4	104.12±13.64^{a}	94.83±8.67^{a}	157.02±11.79^{b}
8	92.95±10.87^{a}	129.59±25.93^{b}	176±32.07^{c}
24	145.29±29.73^{b}	93.40±10.63^{a}	210.14±49.36^{c}

Note: Different superscript notations indicate significantly different results ($p<0.05$).

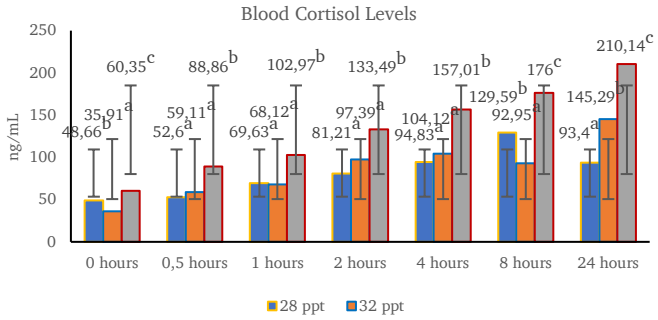

Figure 1. Graph of average blood cortisol during the study.

The results in the table above were then graphed to make it easier to see the increase in cortisol between treatments, and to see how different they were. Cortisol observations in the blood at the beginning showed the highest average blood cortisol levels in the 36 ppt 0.5 hour treatment (57.57 ng/mL), significantly different from the 28 ppt (48.66 ng/mL) and 32 ppt (35.91 ng/mL) treatments. The lowest average blood cortisol levels were in the 32 ppt treatment (35.91 ng/mL). In the 0.5-hour observation, the results showed the highest average cortisol in the 36 ppt treatment (88.86 ng/mL), significantly different from the 28 ppt and 32 ppt treatments, but the 28 ppt treatment (52.6 ng/mL) was not significantly different from the 32 ppt treatment (59.11 ng/mL). The lowest average blood cortisol levels were in the 28 ppt treatment (52.6 ng/mL). In 1 hour, observation showed the highest average blood cortisol in the 36 ppt treatment (102.97 ng/mL), significantly different from the 28 ppt and 32 ppt treatments, but the 28 ppt treatment (69.63 ng/mL) was not significantly different from the 32 ppt treatment (68.12 ng/mL). The lowest average blood cortisol levels were in the 28 ppt treatment (93.4 ng/mL).

The occurrence of ups and downs in cortisol levels in the results is because, according to Wendemeyer (1996), the stress response is divided into three stages. (1) Warning reaction. The pituitary-interrenal axis activates catecholamines and corticosteroid hormones to warn the body. (2) Endurance stage. The physiological system successfully adapts. Energy is needed to replace the immune system and reduce growth so that cortisol levels can experience a decrease in number. (3) Exhaustion stage. The length of time or the amount of exposure to stressors will cause acute stress, and the individual will fight beyond the tolerance limit of adjustment. Physiological

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changes are needed to balance homeostasis. Immune protection becomes weak, and an increase in cortisol levels may occur again.

Different salinities can be stressors that affect stress in cantang grouper fish that live at an optimal salinity of 32 ppt, coupled with the absence of a prior adaptation process. Salinity is the weight in grams of all ions dissolved in 1 kg of seawater if all bromine and iodine are replaced with chlorine in equivalent amounts (Arief, 1984). One of the environmental factors that can affect seed development is the osmotic pressure of the media, which is expressed in the form of salinity parameters, because salinity affects stress and survival in fish (Samuki *et al.*, 2023). The provision of different salinity treatments showed the results of the analysis test showing the interaction of different salinities P0 (32 ppt), P1 (28 ppt) and P2 (36 ppt) for 24 hours of observation giving a significant effect ($P < 0.05$) on cortisol levels in the blood and mucus of cantang grouper fish that live with an optimal salinity of 32 ppt. This shows that each different salinity treatment can affect increasing stress in cantang grouper fish, as indicated by cortisol levels in the blood. Under different salinity treatments can be explained through the physiological stress response of fish to environmental changes, especially salinity, can be osmoregulation. Fish maintain internal homeostasis through osmoregulation. When salinity deviates from the optimal level (32 ppt), the fish must expend more energy to regulate ion balance and water content. This increased metabolic demand triggers a stress response that then increases cortisol.

Table 2. Average blood glucose (mg/dL) of cantang grouper ± SD.

Treatment Time (hours)	Salinity		
	32 ppt	28 ppt	36 ppt
0	35.33±4.68[b]	29.83±1.94[a]	30±1.9[a]
0.5	37.33±4.5[a]	51.67±6.86[b]	50.5±7.31[b]
1	47.33±14.95[a]	68.5±4.18[b]	76.17±8.13[b]
2	49.5±19.23[a]	103.5±12.65[b]	108.33±15.04[b]
4	45±19.11[a]	115.33±7.82[b]	133.67±8.43[c]
8	53±13.31[a]	124.83±4.12[b]	136.83±5.91[c]
24	58.5±13.94[a]	124.5±5.13[b]	140.67±8.94[c]

Note: Different superscript notations indicate significantly different results ($p<0.05$).

The results of blood glucose measurements showed an increase along with the increase in the amount of cortisol in the blood, especially in the 36 ppt salinity treatment, which indicated a state of stress in the fish. In the control salinity treatment of 32 ppt, the results were still within normal limits. Then there was an increase starting from the observation of hours 0.5 to 24 hours in the 36 ppt treatment. While in the 28 ppt treatment, there was also an increase from 0.5 hours to 8 hours, then decreased slightly at 24 hours. This happened because the cantang grouper had adapted. According to Xing *et al.* (2019), cantang grouper fish live optimally at a salinity of 32 ppt. Normal blood glucose in cantang grouper has a range of 27.8–47.2 mg/dL (Angwarmas *et al.*, 2020).

Under stressful conditions, fish need energy to maintain homeostatic conditions. The effects of these activities can cause increased metabolism, which has an impact on increasing blood glucose levels. Increased metabolism results in less energy available for growth. High blood glucose levels are caused by the breakdown of muscle glycogen into glucose as a quickly available energy source. Different salinity treatments affect fish blood glucose levels (Hertika *et al.*, 2021). High or low blood glucose values at each hour of treatment are caused by high levels of catecholamine and cortisol hormones due to the chemical stressor response in the form of high and low salinity.

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Table 3. Average of erythrocytes (x 10^6 cells/mm^3) in cantang grouper fish at different media salinities.

Treatment Time (hours)	Salinity		
	32 ppt	28 ppt	36 ppt
0	0.93 ± 0.19^a	0.65 ± 0.11^b	1.03 ± 0.27^a
0.5	0.71 ± 0.08^b	0.72 ± 0.10^b	0.96 ± 0.14^a
1	1.05 ± 0.11^a	0.93 ± 0.12^{ab}	0.87 ± 0.09^b
2	1.04 ± 0.14^a	1.01 ± 0.13^a	0.73 ± 0.07^b
4	0.76 ± 0.11^a	0.74 ± 0.08^a	0.91 ± 0.05^b
8	0.81 ± 0.07^b	0.78 ± 0.07^b	1.02 ± 0.18^a
24	0.83 ± 0.08^b	0.77 ± 0.08^b	1.03 ± 0.27^a

Note: Different superscript notations indicate significantly different results ($p<0.05$).

Observation and calculation of the blood profile of cantang grouper fish in different media salinity begins with the calculation of the number of erythrocytes. A decrease in the number of erythrocytes in fish can indicate anemia or a lack of blood. Changes in red blood cells can be used as a strong indicator of stress with suboptimal salinity, or the presence of toxic substances or pollutants in the aquatic environment (Setiawati *et al.*, 2017). The results of the observation showed that the average number of erythrocytes fluctuated, although it was still within the normal range of fish erythrocytes, namely 20.000 – 3.000.000 cells/mm^3. The increase in the number of erythrocytes was caused by the fish being under stress. When stressed, the blood in the spleen will be pumped into the blood vessels (Wedemeyer and Yutsuke, 1997).

A high erythrocyte count indicates that the fish is hypotonic, meaning that the fluid in the fish's body is higher than the fluid outside its body, so that the red blood cells swell. Observation and calculation of the blood profile of cantang grouper fish at different media salinities is the next step in calculating the number of leukocytes. The average leukocyte cells also increased and decreased, although the value is still within the normal range, namely 20.000 – 150.000 cells/mm^3 (Sani *et al.*, 2014).

Table 3. The results of the calculation of the average number of leukocytes (x 10^5 cells/mm^3) of cantang grouper fish at different media salinities.

Treatment Time (hours)	Salinity		
	32 ppt	28 ppt	36 ppt
0	0.65 ± 0.08^a	0.65 ± 0.11^a	0.65 ± 0.08^a
0.5	0.77 ± 0.07^a	0.54 ± 0.09^b	0.54 ± 0.09^b
1	0.58 ± 0.08^b	0.60 ± 0.06^b	0.60 ± 0.06^b
2	0.57 ± 0.05^b	0.68 ± 0.12^{ab}	0.68 ± 0.12^{ab}
4	0.32 ± 0.05^b	0.50 ± 0.06^a	0.50 ± 0.06^a
8	0.50 ± 0.10^a	0.55 ± 0.10^a	0.55 ± 0.10^a
24	0.57 ± 0.09^a	0.70 ± 0.09^a	0.70 ± 0.09^a

The average leukocyte cell also increased and decreased; however, the value is still within the normal range, namely 20.000 – 150.000 cells/mm^3. Leukocytes are blood cell components that function in the fish's defense system. Decreased leukocytes can be caused by fish experiencing hypertonic meaning that the fluid in the fish's body is higher when compared to the fluid outside its body so that white blood cells experience bulging, not only that, a decrease in the number of leukocyte cells also indicates that the fish's defenses are weakened and there is a delay in coagulation when the fish is injured in a new environment (De *et al.*, 2019).

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Table 4. Results of Differential Leukocyte Calculation (%) of Cantang Grouper Fish at Different Media Salinities.

Salinity	Treatment Time (hours)	Differential Leukocyte				
		Lymphocytes	Neutrophils	Monocytes	Eosinophils	Basophils
32 ppt (P0)	0	81.6 ± 2.4[a]	7.3 ± 1.3[b]	5.0 ± 1.0[b]	4.1 ± 0.7[a]	1.8 ± 0.7[b]
	0.5	85.3 ± 2.1[a]	7.6 ± 1.6[b]	4.0 ± 0.8[b]	2.0 ± 0.8[b]	1.0 ± 0.8[b]
	1	83.0 ± 2.8[b]	9.5 ± 1.3[b]	4.0 ± 0.8[b]	2.0 ± 1.4[b]	1.3 ± 0.8[b]
	2	81.6 ± 3.7[a]	9.0 ± 1.7[b]	5.0 ± 1.0[b]	2.3 ± 1.2[b]	2.0 ± 0.6[a]
	4	84.1 ± 2.2[a]	7.5 ± 1.5[b]	4.1 ± 0.7[b]	1.8 ± 0.7[b]	2.3 ± 0.8[a]
	8	83.5 ± 3.2[a]	8.1 ± 1.9[b]	3.8 ± 0.7[b]	3.0 ± 1.2[a]	1.3 ± 0.5[b]
	24	83.8 ± 3.3[a]	8.0 ± 1.8[b]	4.1 ± 1.3[b]	2.0 ± 0.6[b]	2.0 ± 0.8[a]
28 ppt (P1)	0	73.0 ± 5.6[b]	12.3 ± 2.7[a]	7.8 ± 1.9[a]	4.1 ± 1.1[b]	2.6 ± 0.8[a]
	0.5	77.6 ± 4.2[b]	12.5 ± 3.2[a]	6.0 ± 2.0[a]	1.5 ± 1.0[b]	2.3 ± 0.8[a]
	1	77.5 ± 3.9[a]	11.6 ± 1.8[a]	6.1 ± 2.1[a]	3.5 ± 1.3[a]	1.1 ± 0.7[b]
	2	83.1 ± 3.3[a]	8.1 ± 1.7[b]	4.0 ± 1.0[b]	4.3 ± 1.0[a]	1.0 ± 0.9[b]
	4	77.8 ± 3.1[b]	13.0 ± 2.8[a]	5.6 ± 0.8[a]	2.6 ± 0.8[b]	1.0 ± 0.8[b]
	8	78.1 ± 2.9[b]	12.5 ± 2.5[b]	5.3 ± 1.0[b]	3.3 ± 0.8[a]	1.6 ± 0.8[b]
	24	76.8 ± 4.4[b]	13.0 ± 3.5[b]	6.5 ± 1.6[b]	2.6 ± 0.8[b]	1.0 ± 0.6[b]
36 ppt (P2)	0	80.8 ± 2.3[a]	9.1 ± 1.4[b]	6.0 ± 1.5[b]	2.8 ± 0.7[a]	1.1 ± 0.7[b]
	0.5	77.6 ± 3.0[b]	10.1 ± 2.4[b]	6.8 ± 1.4[a]	3.0 ± 0.8[a]	2.3 ±0.8[a]
	1	84.0 ± 2.8[a]	7.6 ± 1.6[b]	4.3 ± 1.0[b]	1.1 ± 0.7[a]	2.6 ± 1.3[a]
	2	76.1 ± 3.6[b]	14.1 ± 2.6[a]	5.8 ± 0.7[a]	3.5 ± 1.0[b]	0.6 ± 0.1[b]
	4	78.6 ± 3.5[b]	10.0 ± 2.8[b]	5.6 ± 1.5[a]	3.3 ± 0.8[a]	2.3 ± 0.8[a]
	8	82.3 ± 3.2[a]	10.3 ± 3.0[a]	4.0 ± 1.0[a]	0.8 ± 0.1[b]	2.5 ± 1.0[a]
	24	82.3 ±3.5[a]	8.6 ± 2.7[a]	5.1 ± 1.1[a]	3.1 ± 0.7[a]	0.8 ± 0.1[b]

Leukocyte differential is an indicator of immune response as a body defense system. Leukocyte differential observed in this study was lymphocytes, neutrophils, monocytes, eosinophils, and basophils. Based on the results of the study, it is known that lymphocyte cells have the highest percentage compared to other cells. This is related to the role of lymphocytes as producers of immune substances. Different media salinity affects neutrophil cells. The normal percentage of neutrophils in fish blood is 6-8% (Sharma and Langer, 2014). The increase in the percentage of neutrophils can be caused by increased cortisol levels in the body of fish experiencing stress. High cortisol levels in the body can trigger the release of neutrophil cells into the bloodstream by bone marrow (Salim *et al.*, 2016).

The increase in monocytes is caused by the salinity of the fish's living medium not being optimal, causing physiological disorders, namely stress in the fish (Sakai, 1999). The decrease in the percentage of monocytes can be caused because monocytes, as macrophages, do not need much to phagocytize, because the fish do not experience stress that stimulates monocyte production. Similar to neutrophil cells, monocytes are also short-lived, so their number in the blood fluctuates. An increase in the number of eosinophils is called eosinophilia, which describes fish in a state of stress due to less than optimal water quality, and also describes the presence of chronic disease. A decrease in the number of eosinophils can indicate that the fish are infected with a disease and are experiencing acute disease (Sharma and Langer, 2014). The average results of the percentage of basophils in cantang grouper fish at different media salinities ranged from 0 – 2%. These results indicate that the number of basophils has a percentage value above normal.

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CONCLUSION

The difference in salinity (stressor) significantly affects the physiological response, namely an increase in cortisol levels, blood glucose, and hematological responses in the form of an increase in erythrocytes in the blood of cantang grouper (*Epinephelus lanceolatus* x *Epinephelus fuscoguttatus*) for 24 hours. Before the 36 ppt salinity treatment, the average cortisol value was 57.57 ng/mL, and after 24 hours of treatment, the average cortisol value became 210.14 ng/mL. This change in cortisol levels, blood glucose, and hematology in fish can be used as an indicator of stress due to changes in salinity.

CONFLICT OF INTEREST

The conflict of interest contains a declaration that there is no conflict of interest among all authors upon writing and publishing the manuscript.

AUTHOR CONTRIBUTION

The authors' contributions to this study were Anastasya Dewi Larasati, Langgeng Widodo, Melinda Kusuma Ningrum, Ridwansyah, and Fitria Karunia, who managed the sampling, laboratory work, analysis, and writing of the article. Laksmi Sulmartiwi, Gunanti Mahasri, Rr. Juni Triastuti and Lailatul Lutfiyah contributed to the writing of the article and conducted statistical and data analysis.

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