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The Mitogen-Activated Protein Kinase (MAPKs) Expression and Primary Stress Hormone (Cortisol) of *Clarias* sp. Juvenile in Different Light Intensity

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Abstract

Clarias sp. is one of the major genera of freshwater catfish and also a common commodity in tropical aquaculture. Excessive light intensity is expected to be responded to by fish as environmental stressors. Fish that experience stress will increase the secretion of catecholamine, cortisol, blood glucose levels, and p38 MAPKs in catfish. The different intensities of light will affect the fish's physiological processes. The purpose of this study was to analyze the physiological responses (Level of blood glucose, primary stress hormone (Cortisol) and p38 MAPK$_s$) of catfish *Clarias* sp. which were incubated at different lights intensity of catfish. 300 fish were collected from Kepanjen Regency, East Java. Blood and meat sampling are carried out to test the profile of glucose, cortisol, and p38 MAPKs. The experimental research at this stage was carried out by transferring the catfish from Kepanjen to an experimental aquarium (50 x 30 x 30 cm) that set the different light intensities (0 lux (control), 400 lux, 800 lux, and 1200 lux). Each experimental aquarium is filled with 15 catfish and will be kept for 21 days. Quantification of the parameter using ELISA method. The study result showed the highest peak of primary stress hormone (cortisol) levels is 34±1,50 ng/mL, blood glucose levels are 120±3,4 mg/dL, and p38 MAPK is 97±1,1 %. All of it was achieved in the treatment of 1200 lux in the 2nd week.

INTRODUCTION

Over the last few years, the demand for catfish in Indonesia is the second largest. In 2017, the national production of Catfish was 1,125,526 tons; in 2018, it was 1,027,195 tons. Based on the latest data recorded by the Indonesian Central Statistics Agency, the total production of catfish in Indonesia in 2019 was the second largest after tilapia, which was 1,000,647 tons (BPS, 2018). In 2020, the Indonesian Ministry of Maritime Affairs and Fisheries targets catfish production of 1.49 million tons, or an increase of 16.8% (KKP, 2020). Dumbo catfish rarely show their activity and prefer a cool and dark atmosphere. *Clarias* sp. are nocturnal (active at night). They look for food usually at night (Hadiaty and Yamahira, 2014).

Lights are one of the most important factors for growth that includes the color spectrum, photoperiod, and intensity (Fajarwati, 2006; Kang *et al.*, 2013). In general, high intensity of light will further

optimize growth, but the intensive intensity of light can cause stress to fish and even death (Nofrizal *et al*., 2009; Abdel-Rahim *et al*., 2019). Stressed fish will avoid anabolic activities such as growth and reproduction, in the long term can cause a decrease in growth, disease resistance, reproductive success, the appearance of swimming, and characteristics of the biota population (Islam *et al*., 2019). Naturally, the cells of fish respond to the presence of environmental stressors by producing stress hormones such as mitogen-activated protein kinase (MAPKs). MAPKs are one of the important cellular signaling systems in catfish response to the presence of environmental stressors, so this stress hormone has the potential to be used as molecular biomarker (Masjudi *et al*., 2016).

This study aims to analyze physiological responses (blood glucose levels, cortisol hormone, and p38 MAPKs) of *Clarias* sp. juveniles which were maintained at different intensities of lights. The benefit of this study is to provide information about the range of light intensity that can be tolerated by dumbo catfish juveniles seen from stress responses and add insight into science as well as the development of indoor scale catfish cultivation. As well as consideration for creating a new alternative in the field of aquaculture.

METHODOLOGY

Place and Time

This research was conducted from September to October 2020. Research observation at the Laboratory of Fish Reproduction, Fisheries and Marine Sciences Faculty, Brawijaya University, Malang. Analysis of fish blood glucose, cortisol hormones, and p38 MAPKs was carried out at FAAL Laboratory, Medicine Faculty, Brawijaya University.

Research Materials

The test animals used were catfish (*Clarias* sp.) purchased from an aquaculturist in Kepanjen Regency, East Java. The materials used were Eppendorf tube, EDTA, commercial enzyme kit (Glu L1000, PLIVA-Lachema, Czech Republic), glucose oxidase solution, peroxidase, 4-amino antipyrine, ELISA Fish Cortisol Kit (E0014FI, BT. LAB), paraffin block, xylene, 100% ethanol, 95% ethanol, 70% ethanol, anti-p38 monoclonal antibody, phosphate buffer saline (PBS), and DAB chromogen (Diaminobenzinidine).

The equipment used during this study were lights with various intensities (0 lux (control), 400 lux, 800 lux, and 1200 lux), an experimental aquarium (size 60x30x25cm), aeration, spuit, sectio set, 96-well microtiter plate (200 µl), plate reader (Tecan Sunrise, USA), and light microscope.

Research Design

The experimental design in this study used a randomized design Complete (CRD) 2 Factors with 4 treatments 3 times treatment provided include (A) Control (0 lux), (B) 400 lux, (C) 800 lux, and (D) 1200 lux. The light intensity value refers to previous research according to (Tian *et al*., 2015), high intensity of light (more than 800 lux) not only impacts poor growth, but may also cause response stress, low light intensity below 400 lux also shows oxidative stress, decreased immune system, and immunosuppression. This research was conducted for 21 days in which sampling intake on days 0, 7, 14. and 21 the measurement of retinal adaptation, glucose blood, Cortisol hormone, p38MAPKS, and on Survival Rate data collection at the beginning and end of the study.

Work Procedure

Animal Preparation

The fish were placed in an acclimatization tank and fed with commercial feed once per day. After 14 days of holding periods, fish were classified. Then they were transferred and acclimatized into an experimental aquarium (size 60x30x25cm) with an

aeration system for 2 days. If the mortality of catfish is less than 3% during 48 hours, it means that the catfish population will be considered worthy of experimental testing. However, if fish mortality is over 3%, the fish should replace with the new population from the acclimatization tank and then reacclimatized for 2 days.

Fish Blood and Meat Sampling

Blood sampling is carried out to observe the profile of blood glucose and cortisol. The fish blood is taken as much as about 0,1 ml using a spuit. Then the blood is stored in the Eppendorf tube.

Figure 1. Fish blood and meat sampling.

Add EDTA 1%. The blood was observed at 0, 7, 14, and 21 days of further handling in the laboratory. The parameters of the study were tested using the ELISA method (Velasco-Santamaría and Cruz-Casallas, 2007). Meat sampling is carried out to test the profile of MAPK. The meat is taken as much as 1 gram with sectio set equipment. Then the meat is stored in the film bottle and added formalin 10%. The parameters of the study were tested using the ELISA method.

Glucose Determination

The level of blood glucose determination using a kitTench plasma glucose concentration was analyzed according to published methods (Bartoňková *et al*., 2016) using a commercial enzyme kit (Glu L1000, PLIVA-Lachema, Czech Republic). Samples were added to a glucose oxidase solution, peroxidase, and 4-amino antipyrine; after 10 min incubation, samples were moved to a 96-well microtiter plate (200 µl) and the plate reader (Tecan Sunrise, USA) measured their absorbance (500 nm). The glucose concentration was consequently determined using the standard absorbance of glucose (10 mmol/l). The quantification limit of this method was 0.021 mmol/l; The working range was 0.065–45 mmol/l, repeatability was 1.05%, and the working volume was 10 µl.

Cortisol Determination

Sampling using catfish juvenile meat as much as 1 gram for 1 sample. Calculation of cortisol hormone levels using the ELISA method (E0014FI, Fish Cortisol Elisa Kit, BT. LAB) based on the published method (Velasco-Santamaría and Cruz-Casallas, 2007). Optical density (OD) readings were taken at a wavelength of 450 nm. This measurement is carried out in the Physiology Laboratory (FAAL) Universitas Brawijaya.

Determination of p38 MAPK Levels

Calculation of glycogen levels using the ELISA modification according to published methods (Luo *et al*., 2020). The first stage is deparaffin preparation (paraffin block) with xylene 3 times for 3 minutes each then rehydrating the preparation using 100% ethanol, 95% ethanol, and 70% ethanol for 2 minutes, 2 minutes, 1 minute and finally with water for 1 minute. The preparations were then immersed in a peroxidase blocking solution at room temperature for 10 minutes and then incubated in diluted blocking serum at 25 °C for 10 minutes. The incubated preparations were then immersed in anti-p38 monoclonal antibody at 25 °C for 10 minutes. After

that, the preparations were washed with phosphate buffer saline (PBS) for 5 minutes. The preparations were incubated with secondary antibodies (conjugated with rat radish peroxidase) at 25 °C for 10 minutes and then washed again with PBS solution for 5 minutes. Next, the preparation was incubated with peroxidase at 25 °C for 10 minutes and then washed with PBS for 5 minutes; incubated with DAB chromogen (Diaminobenzinidine) at 25 °C for 10 minutes; incubated with hematoxylin Eosin for 3 minutes and then washed with running water. After that, the preparation was dripped with mounting media and covered with a cover slip and then observed the expression of p38 (brown color) was in cells using a light microscope with 1000x magnification.

Data Analysis

The data obtained from observations are presented in the form of tables and graphs. The experimental design used a completely randomized design (CRD) with 4 treatments and 3 replications. Furthermore, the data obtained were analyzed using variance analysis (ANOVA) and the F test at a 95% confidence interval. Then to see the difference between the treatments carried out Tukey's (Honestly Significant Difference) test with a confidence interval of 95%.

RESULTS AND DISCUSSION

Cortisol

The results of the study (Figure 2), the lowest week-0 cortisol (μg/mL) level in treatment C was equal to 13.87 ± 0.75 and the highest in the control treatment (A) which was 14.93 ± 2.02. There were no significant differences between treatments, with sig values> 0.05 (p = 0.842) in the first week. The lowest at the control treatment (A) is equal to 16.53 ± 1.7 in the first week and the highest at treatment D is equal to 28.40 ± 2.64 there are significant differences between treatments, values (p <0.05). The lowest at the control treatment (A) is equal to 20.73 ± 6.5 in the second week and the highest at treatment D is equal to 34.27 ± 1.50 there are significant differences between treatments, values (p <0.05). The lowest was at the control treatment (A) which was 17.53 ± 2.94 in the third week and the highest was at treatment D which was 33.67 ± 2.20 there were significant differences between treatment values (p <0.05).

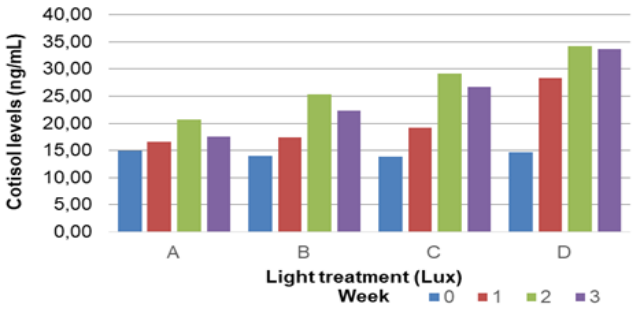

Figure 2. Cortisol level of catfish during the research.

Based on the results of the above observations, it was shown that different light-intensity treatments could increase cortisol (stress hormone) levels and have significant differences then the 0 lux treatment (control) (p <0.05). Treatment D with the highest light showed significant differences (p <0.05) for treatments B and C. It can be concluded that higher light treatment can increase cortisol levels in Catfish (*Clarias* sp.).

Plasma cortisol is an important stress hormone and it was produced through the hypothalamic-pituitary-interrenal axis after external stimulation. The plasma concentration of cortisol can

be seen as a stress signal in fish (Sadoul and Geffroy, 2019). When the light intensity is too high, it can cause stress and death. The high levels of circulating catecholamines and cortisol will trigger a secondary response involving metabolic physiology. Both of these phases are adaptive and fish can adjust themselves to stressors and able to maintain homeostasis (Gans and Coffman, 2021).

Glucose

The results of the study (Figure 3), the lowest glucose level (mg/dL) in week 0 in the control treatment (A) was 34.67 ± 3.05, and the highest in treatment D was 38.33 ± 2.08 with no significant difference between treatment, sig value> 0.05 (p = 0.092). The lowest first week in the control treatment (A) is equal to 41 ± 1 and the highest in treatment D is equal to 95.67 ± 4.04 there are significant differences between treatments, values (p <0.05). The second week is lowest in the control treatment (A) which is equal to 49 ± 6.5 and the highest at treatment D which is equal to 120 ± 3.4 there are significant differences between treatments, values (p <0.05). The third week is the lowest in the control treatment (A) is equal to 43.0 ± 1.7 and the highest in treatment D is equal to 88.3 ± 4.5 there are significant differences between treatments, and the value (p <0.05).

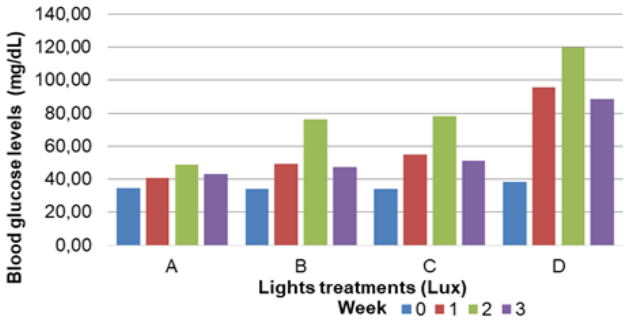

Figure 3. Glucose level of catfish during the research.

Based on the results of the above observations, it is shown that different light treatments increase glucose levels at the beginning and decrease them in the 3rd week. D treatment was significantly different from the control treatment (A) (p <0.05). The highest D treatment with D also showed a significant difference (p <0.05) in glucose results in groups B and C.

Normal fish blood glucose levels contain 40-90 mg/dL (Malini *et al*., 2018). It can be concluded that higher light treatment has increased blood glucose so that Catfish (*Clarias* sp.) is stressed. When the fish experiences a disturbance that causes stress, the fish body will issue a sign or alarm as an indication of a disturbance. Alarms in fish include an increase in blood sugar due to hormone secretion from the adrenal gland. A supply of sugar, such as glycogen in the liver, is metabolized as an energy supply for emergencies. Blood glucose is the main source of energy and an essential substrate for cell metabolism especially the brain cells in the fishes.

The continuous increase in blood glucose levels indicates the flow of glucose into the blood, which is greater than the entry of blood glucose into the cell. Conversely, glucose levels will decrease if the flow of glucose into the blood is lower than the entry of blood glucose into the cell. Thus, the peak of blood glucose level occurs when the flow of glucose into the blood and the entry of blood glucose into the cell reaches its equilibrium point. Glucose that has entered the cell will be metabolized immediately to meet energy needs and avoid using several amino acids as a source of metabolic energy (Svobodová *et al*., 2003). Rapid increases in blood glucose levels can trigger insulin bioactivity at the highest level; so, the

inclusion of blood glucose into cells, takes place rapidly (Antony and Vijayan 2021).

P38 MAPK

The results of the study (Figure 4), p38 of the third week's MAPKs was lowest in treatment A (control), that IHK (%) is 59.23 ± 3.57, and the highest was in treatment D, which was IHK (%) is 97.3 ± 1.1 there were significant differences between treatments B and C, with values ($p < 0.05$). The increase in the percentage of p38 MAPKs shows that catfish experience stress and Catfish detect light intensity as a stressor.

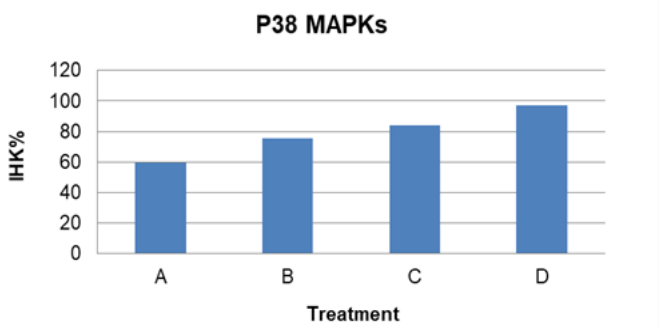

Figure 4. The P38 MAPKs of catfish during the research.

This happens because of ROS activation, then ROS will activate p38 MAPKs. Activation of the p38 MAPKs enzyme is known to be induced by various endogenous and exogenous stress stimuli, including hyperglycemia, ROS, oxidative stress, osmotic stress, heat shock, and light radiation (Rose *et al*., 2010). Naturally, cells in fish respond to the presence of environmental stressors by producing stress proteins such as mitogen-activated protein kinases (MAPKs) (Fredo *et al*., 2020).

The p38 MAPKs enzyme is a member of the MAP family of serine/threonine protein kinases, in addition to extracellular signal-regulated protein kinases (ERK1 or p44MAPK), ERK2 (P42MAPK) and C-JUN NH2-terminal kinases (JNK) or stress-activated protein kinases (SAPK). ROS can activate ASK1 which is a sensor of oxidative stress. ASK1 activates MKK3 / 6 (MAPK kinase) which subsequently phosphorylates the p38MAPK enzyme at threonine 180 and tyrosine 182 so that the p38 MAPK enzyme becomes active. The activated P38 MAPK enzyme can phosphorylate the transcription factor (ATF-2) activator which stimulates gene transcription. The active p38 MAPK enzyme can affect several cellular processes, including cell growth and apoptosis, inflammation and stress-specific tissue responses through the regulation of gene expression (Zeyen *et al*., 2022).

CONCLUSION

Lights intensity affects the physiological response (cortisol, blood glucose and p38 MAPKs) of catfish (*Clarias* sp.). High levels of cortisol, blood glucose and p38 MAPK indicate stressful fish.

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REFERENCES

Abdel-Rahim, M.M., Lotfy, A.M., Aly, H.A., Sallam, G.R. and Toutou, M.M., 2019. Effects of light source, photoperiod, and intensity on technical and economic performance of meagre, *Argyrosomus regius*, on intensive land- based farms. *AACL Bioflux*, *12*(5), pp.1531–1545. http://www.bioflux.com.ro/docs/2019.1531-1545.pdf

Antony, P. and Vijayan, R., 2021. Bioactive peptides as potential nutraceuticals for diabetes therapy: A comprehensive review. *International Journal of Molecular Sciences*, *22*(16), 9059. https://doi.org/10.3390/ijms22169059

Bartoňková, J., Hyršl, P. and Vojtek, L., 2016. Glucose determination in fish plasma by two different moderate methods. *Acta Veterinaria Brno*, *85*(4), pp.349–353. http://dx.doi.org/10.2754/avb201685040349

[BPS] Badan Pusat Statistik., 2018. *Produksi perikanan budidaya menurut komoditas utama (ton), 2018*. https://www.bps.go.id/indicator/56/1513/2/produksi-perikanan-budidaya-menurut-komoditas-utama.html

Fajarwati, E.N., 2006. *Aspek eko-biologi ikan lalawak (Barbodes balleroides) pada berbagai ketinggian tempat di Kabupaten Sumedang, Jawa Barat*. Institut Pertanian Bogor, Bogor. p.50. https://repository.ipb.ac.id/handle/123456789/45871.

Fredo, S., Widodo, M.S. and Armando, E., 2020. The effect of different temperature on survival rate and P38 Mapk (mytogen activity protein kinase) Of *Pangasius djambal*. *Journal of Aquaculture Development and Environment*, *3*(1), pp.148-151. http://dx.doi.org/10.31002/jade.v3i1.2357

Gans, I.M. and Coffman, J.A., 2021. Glucocorticoid-mediated developmental programming of vertebrate stress responsivity. *Frontiers in Physiology*, *12*, 12195. https://doi.org/10.3389/fphys.2021.812195

Hadiaty, R.K. and Yamahira, K., 2014. The loaches of the genus Nemacheilus (Teleostei: Nemacheilidae) in Sunda Islands, with an identification key. *Jurnal Iktiologi Indonesia*, *14*(2), pp.83-100. https://doi.org/10.32491/jii.v14i2.85

Islam, M.A., Uddin, M.H., Uddin, M.J. and Shahjahan, M., 2019. Temperature changes influenced the growth performance and physiological functions of Thai pangas *Pangasianodon hypophthalmus*. *Aquaculture Reports*, *13*, 100179. https://doi.org/10.1016/j.aqrep.2019.100179

Kang, J.H., KrishnaKumar, S., Atulba, S.L.S., Jeong, B.R. and Hwang, S.J., 2013. Light intensity and photoperiod influence the growth and development of hydroponically grown leaf lettuce in a closed-type plant factory system. *Horticulture, Environment, and Biotechnology*, *54*(6), pp.501–509. https://doi.org/10.1007/s13580-013-0109-8

[KKP] Kementerian Kelautan dan Perikanan., 2020. *Komoditas lele jaid primadona masyarakat, KKP pacu produksi nasional melalui sistem bioflok*. https://kkp.go.id/djpb/artikel/28416-komoditas-lele-jadi-primadona-masyarakat-kkp-pacu-produksi-nasional-melalui-sistem-bioflok

Luo, Z., Huang, W., Wang, G., Sun, H., Chen, X., Luo, P., Liu, J., Hu, C., Li, H. and Shu, H., 2020. Identification and characterization of $p38^{MAPK}$ in response to acute cold stress in the gill of Pacific white shrimp (*Litopenaeus vannamei*). *Aquaculture Reports*, *17*, 100365. https://doi.org/10.1016/j.aqrep.2020.100365

Malini, D.M., Madihah, Apriliandri, A.F. and Arista, S., 2018. Increased blood glucose level on pelagic fish as response to environmental disturbances at East Coast Pangandaran, West Java. *IOP Conference Series: Earth and Environmental Science*, *166*, 012011. https://doi.org/10.1088/1755-1315/166/1/012011

Masjudi, H., Tang, M.U. and Syawal, H., 2016. Kajian tingkat stres ikan tapah (*Wallago leeri*) yang dipelihara dengan pemberian pakan dan suhu yang berbeda. *Berkala Perikanan Terubuk*, *44*(3), pp.69–83.

http://dx.doi.org/10.31258/terubuk.44.3.69%20-%2083

Nofrizal, Yanase, K. and Arimoto, T., 2009. Effect of temperature on the swimming endurance and post-exercise recovery of jack mackerel *Trachurus japonicus* as determined by ECG monitoring. *Fisheries Science*, 75, pp.1369–1375. http://dx.doi.org/10.1007/s12562-009-0164-3

Rose, B.A., Force, T. and Wang, Y., 2010. Mitogen-activated protein kinase signaling in the heart: Angels versus demons in a heart-breaking tale. *Physiological Reviews*, *90*(4), pp.1507–1546. https://doi.org/10.1152/physrev.00054.2009

Sadoul, B. and Geffroy, B., 2019. Measuring cortisol, the major stress hormone in fishes. *Journal of Fish Biology*, *94*(4), pp.540–555. https://doi.org/10.1111/jfb.13904

Svobodová, A., Psotová, J. and Walterová, D., 2003. Natural phenolics in the prevention of UV-induced skin damage: a review. *Biomedical Papers*, *147*(2), pp.137–145. https://doi.org/10.5507/bp.2003.019

Tian, H.Y., Zhang, D.D., Xu, C., Wang, F. and Liu, W.B., 2015. Effects of light intensity on growth, immune responses, antioxidant capability and disease resistance of juvenile blunt snout bream *Megalobrama amblycephala*. *Fish & Shellfish Immunology*, *47*(2), pp.674–680. https://doi.org/10.1016/j.fsi.2015.08.022

Velasco-Santamaría, Y. and Cruz-Casallas, P., 2007. Methodology for determination of plasma cortisol in fish using competitive enzyme-linked immunosorbent assay (ELISA). *Revista MVZ Córdoba*, *12*(1), pp. 869–877. http://www.scielo.org.co/scielo.php?script=sci_arttext&pid=S0122-02682007000100002

Zeyen, L., Seternes, O.M. and Mikkola, I., 2022. Crosstalk between p38 MAPK and GR Signaling. *International Journal of Molecular Sciences*, *23*(6), 3322. https://dx.doi.org/10.3390%2Fijms23063322