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Correlation Between Glucose Level And Protozoan Ectoparasite Infestation Level Of Humpback Grouper (*Cromileptes altivelis*) Nursery In UPBL Situbondo, East Java

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Abstract. The purpose of this study was to determine the correlation between blood glucose levels of humpback grouper with an infestation of protozoa ectoparasites and the correlation between blood glucose levels and the infestation of protozoa ectoparasites in humpback grouper (*Cromileptes altivelis*) in the Sea Cultivation Development Unit, Situbondo, East Java. This research is survey research. The sample was in the form of a humpback grouper (*Cromileptes altivelis*) measuring 13-20 cm, 30 of which were randomly drawn from a population of 2,000 individuals. The results of the study showed that the blood glucose level of the humpback grouper Nurserys was the lowest of 21 mg / dL and the highest was 67 mg / dL. Humpback grouper Nurserys began to be infested with *Trichodina* sp. Ectoparasites, and *Cryptocaryon* sp. in fish with glucose levels of 25-28 mg / dL included in the category of normal infestations, while fish with glucose levels of 29 - 36 mg / dL (Normal) are included in the category of mild to severe infestations. The results showed that there was a very close correlation between blood glucose levels of humpback grouper Nurserys and ectoparasite infestations. The higher the blood glucose level the higher the ectoparasite infestation in the Nurserys of the humpback grouper.

1. Introduction

Commodity Grouper fish production in 2015 was 8,972 tons and increased by 15,089 tons in the third quarter of 2016 [1]. Market demand for groupers comes from domestic and foreign (export). Exports grouper in Indonesia in 2016 amounted to 32.18 million US \$, until July 2017 it reached 16.42 million US \$. The export volume in the same period continues to increase by an average of 30.75% per year [2].

Many factors often cause failure in producing humpback grouper seeds, both in hatcheries and in KJA, among others are poor quality larvae and inappropriate management of water quality. A good water environment as a living medium for fish can increase the growth and physiological conditions of fish. The physiological conditions of fish can be identified by blood characteristics. Blood can be used to evaluate the physiological response in fish, including stress responses. The stress response in animals can be seen from changes in hormone levels of cortisol, blood glucose, hemoglobin, and hematocrit [3].



An unfavorable environment and stressful fish conditions will cause the body's defenses to decline and fish to be weak, thus giving an opportunity to pathogens both parasites, bacteria, fungi and viruses to develop well because the environment is in accordance with their needs. One of the diseases that often arise in grouper cultivation is a disease caused by parasites [4]

According to Cruz-Lacierda [5], there are several types of parasitic diseases including grouper protozoa, monogenea, nematodes, isopods, and sea leeches. Diseases that attack groupers from phylum protozoa include Amyloodioniosis, Cryptocaryonosis, Trichodiniosis, Brooklynelliosis, Renal sphaerosporosis, and Microsporidiosis. This parasite infestation can increase stress levels in fish. Based on these data, a correlation between blood glucose levels and protozoa infestation should be conducted on the seeds of humpback grouper fish.

The objectives of this study are as follows: 1) To measure blood glucose levels in humpback grouper (*Cromileptes altivelis*), 2) To calculate the level of protozoa infestation in humpback grouper (*Cromileptes altivelis*) and 3) To determine the correlation between blood glucose levels and level of infestation of protozoa in humpback grouper (*Cromileptes altivelis*).

The benefit of this study is to provide information about the correlation between blood glucose levels and the level of infestation of protozoa in humpback grouper (*Cromileptes altivelis*) so that it can be used as a reference in mouse grouper aquaculture activities.

2. Research methodology

2.1. Research method

This research was conducted at the Education Laboratory of the Faculty of Fisheries and Marine Affairs, Airlangga University, Surabaya in October - December 2018. The method used in the study was a survey method, namely by taking samples from a population-based on actual facts.

2.2. Sample collection

Sampling is done randomly where all the seeds in the tub have the same opportunity to be taken. According to Cameron (2002) the minimum number of samples that can be represented in survey research with a population greater than 1000 is 27. Therefore the number of samples in this study was 30 individuals.

2.3. Measurement of blood glucose levels

Measurement of glucose levels begins with taking blood using a 1 ml syringe that has been moistened using a 10% solution of ethylene diamine tetra acetate (EDTA) as an anticoagulant. Blood samples are taken from the caudal / fish tail, namely the caudal vein. The blood that has been taken is then inserted at the end of the test strip that has been inserted in the digital blood glucose test kit EasyTouch brand and then a number will show the blood glucose level. The results obtained are recorded.

2.4. Protozoa examination

Protozoa examination is carried out by the native method that is without using staining using a microscope. The inspection was carried out on the surface of the body of the mouse grouper and gill. The surface of the body is scraped and then the scraping results are placed on a glass object, covered with a glass cover and added physiologically NaCl (NaCl 0.85%). Gills are taken and placed on a glass object, then dropping physiological NaCl and then covered with a glass cover. Furthermore observed using a microscope with magnification of 100x and 400x.

2.5. Data analysis

Data on the results of blood glucose examination and ectoparasite infestations in rat grouper fish are presented in the form of figures and tables and are described. To determine the correlation between blood glucose levels and ectoparasite infestations using simple correlation analysis (Steel and Torrie, 1993).

3. Results and discussion

The results of protozoa ectoparasite examination in 30 heads of grouper fish samples taken from the Situbondo UPBL showed that there were 17 positively infested protozoa ectoparasites, namely from Genus *Trichodina* and *Cryptocaryon*. High and low glucose levels are one indicator that can be used to determine the stress level of grouper seeds. According to Poerchas [6] that the normal glucose level of fish is 28.8 - 34.4 mg / dL. For more details, the average glucose levels, infestations, and infestation levels can be seen in Table 1.

Table 1. Results of Measurement of Blood Glucose Levels and Calculation of Ectoparasites in Humpback Grouper Seeds

Blood Glucose Level (mg/dL)	Σ Ectoparasite	Infestation Degree
21–24	0	Normal
25–28	0 – 5	Normal – Low
29–36	17–32	Moderate
37–57	17–57	Moderate – Heavy

Table 1 shows that the mouse grouper seed fish that have below normal blood glucose levels which are below 28 mg / dL are not infested by ectoparasites. The infestation of ectoparasites began to occur on grouper fish with blood glucose levels of 25-28 mg/ dL with infestations of 0 - 5 parasites per tail with normal degrees of infestation - mild. Normal glucose levels in fish range from 28.8 - 34.4 mg / dL [7]. Table 5.1 also shows that in the condition of grouper seeds with normal levels of 29-36 mg/ dL the ectoparasites were infested with moderate degrees of infestation. Furthermore, it was also seen that grouper seeds with normal glucose levels, namely 30-36 mg/ dL grouper fish were also infected with ectoparasites between 17 - 32 ectoparasites, so that they were included in the medium category. Whereas the glucose level above normal is 37 - 57 mg/ dL grouper fish infested by an average of 17-57 ectoparasites included in the medium-heavy category.

To determine the closeness of the correlation between blood glucose levels and ectoparasite infestations, the data were analyzed using Statistical Package for Social Science (SPSS) with correlation analysis. The results of the analysis showed that between blood glucose levels and ectoparasite infestations obtained an r-value of 1, which can be interpreted that the closeness of the relationship between blood glucose levels of grouper seeds and the degree of ectoparasite infestation of the correlation was very high, with a 0.01 confidence.

For more details, a graphical picture of the correlation between glucose levels and ectoparasitic infestation of humpback grouper seeds can be seen in Figure 1.

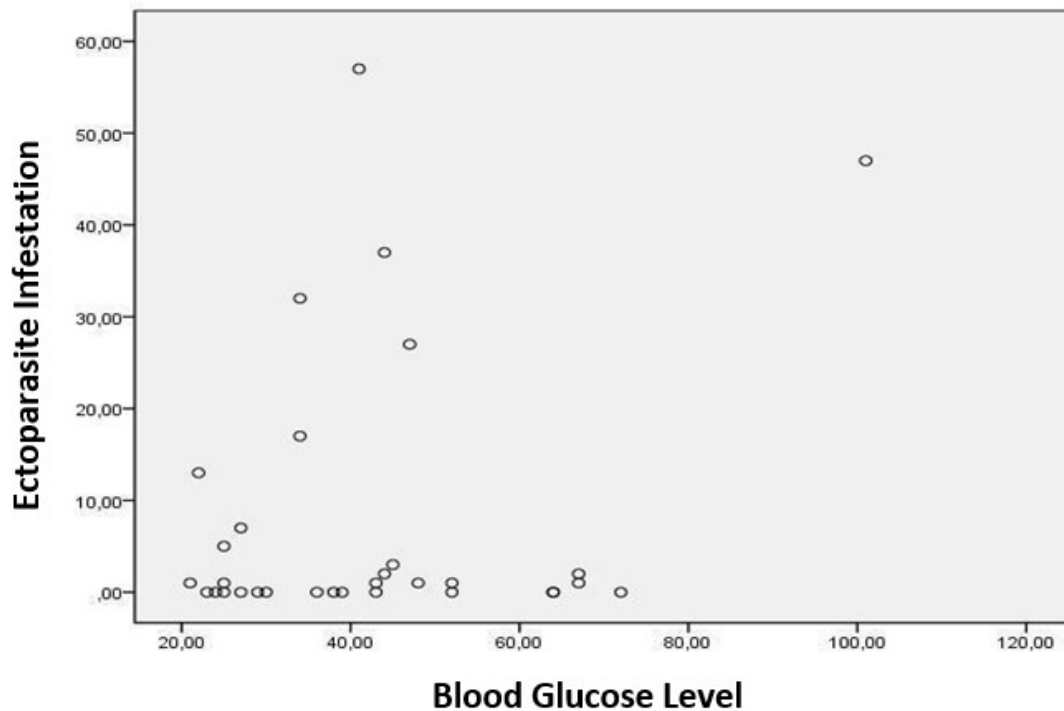


Figure 1. Graph of correlation between blood glucose level and ectoparasite infestation.

The results showed that there was a high degree of closeness between the blood glucose level and ectoparasite infestation on humpback grouper seeds taken from UPBL Situbondo, East Java ($p < 0.01$). Based on the graphs the results of statistical tests show that the correlation between blood glucose levels and ectoparasite infestation (Figure 1) shows that the increase in glucose levels is in line with the increase in ectoparasite infestation in the seeds of the mouse grouper. This can be interpreted that the higher the blood glucose level of the mouse grouper fish, the higher the ectoparasite infestation. This is in accordance with the research conducted by Sulmartiwi [8], that stressed fish conditions are characterized by an increase in blood glucose levels which can result in spurring parasitic infestation in the seeds of humpback grouper fish in concrete tanks at the Hatchery.

The results also showed that protozoa ectoparasites found were *Trichodina* and *Cryptocaryon* which had been identified according to research before [9]. Both of these ectoparasites have been reported by Yanong [10] in humpback grouper. *Trichodina* causes a disease known as *Trichodiniasis* or what is called itching, which causes fish to often rub its body against hard objects around it. Whereas *Cryptocaryon* attacked fish showed clinical symptoms of weakfish, decreased appetite, thinness and shortness of breath.

Stressed grouper fish seeds are shown with high glucose levels because the humpback grouper seeds that are kept in the Hatchery in concrete tanks cause physiological changes in the presence of increased glucose levels. This is because the water temperature in the concrete tub will tend to increase and the grouper habitat that is less suitable is on the coral bottom. That grouper habitat on the coral bottom is at an optimal temperature range of 28–29 °C [11]. Judging from the high levels of ammonia in maintenance media, namely 0.5 - 0.7 and decreased pH which is around 7 can be used as an indicator of the high organic matter in the maintenance medium. This will cause a decrease in water quality and fish will be stressed, which is characterized by high glucose levels.

High glucose levels caused by receptor organs will receive information that will be delivered to the brain of the hypothalamus, then the *kromaffin* cells will secrete the catecholamine hormone. This hormone will suppress the secretion of the hormone insulin which serves to help supply glucose into cells, causing an increase in glucose levels into the blood. This hormone will also activate enzymes involved in catabolism of liver and muscle glycogen deposits. At the same time, the hypothalamus will secrete CRF

(Corticoid Releasing Factor) which will regulate the pituitary gland to secrete ACTH (Adenocortico-Tropic Hormone). ACTH functions to regulate growth mechanisms, cell metabolism, regulation and secrete cortisol. This hormone will regulate the hormone cortisol from interrenal. This cortisol will bully the enzymes involved in gluconeogenesis which results in an increase in blood glucose that comes from noncarbohydrate sources. The occurrence of catabolism of proteins to form glucose also produces amino acids, so that amino acids in the blood increase. Increasing amino acids in the blood will activate insulin again so that it can carry out glucose transport, so that glucose in the blood will decrease again [12].

The grouper seeds that are experiencing stress will cause the body's defense system to decrease and cause fish to be susceptible to ectoparasite infestations so that it can cause high ectoparasite infestations. That changes in glucose levels in fish seeds will weaken the body's resistance [13]. The disease will attack fish if there is an interaction between host, pathogen and environment. These interactions cause stress on fish, so the self-defense mechanism weakens, and causes disease to easily enter the body and cause disease. The results of the study before [14] also added that groupers would be susceptible to parasites due to a decreased immune system.

Seen from Table 1 shows that the lowest sample glucose level of fish is 21 mg / dL and fish have been infected with ectoparasites as much as 1 parasite, but sample fish with glucose levels of 23 mg / dL and some sample fish with higher glucose levels even reach 72 mg / dL is also not infected with ectoparasites. The highest glucose levels occur in grouper fish no. 18 which is equal to 101 mg / dL with a total infestation of 47 parasites, with the degree of infestation showing the moderate category. The highest ectoparasite infestation was 57 parasites included in the heavy category that occurred in grouper seeds with glucose levels of 57 mg / dL. These results indicate that groupers with glucose levels below normal, normal or above normal can all be infected with ectoparasites. This is because fish seeds are kept in the Hatchery with concrete tanks. The temperature of the grouper maintenance media with a concrete base can reach 31 ° C [15], which is also shown in the results of temperature measurements during the first and second samples which range from 28 ° C- 30 ° C. The tendency of low pH and high ammonia levels in maintenance media during maintenance will cause decay due to the accumulation of organic matter at the bottom like a maintenance. This will cause the endurance of the mouse grouper fish to decline and cause ectoparasite infestation to increase. The best water quality is important in the cultivation of fish for quality of water that good ability to provide optimal results on the cultivation of fish. Water quality affected by various chemicals dissolved in water includes temperature, ph, and oxygen dissolved [16].

Based on the results of statistical analysis with a simple correlation obtained the correlation coefficient value $r = 1$ ($p < 0.01$) which can be interpreted that the correlation between blood glucose levels and the level of infestation of ectoparasites has high closeness. This can be interpreted that an increase in glucose levels will be followed by an increase in the level of ectoparasite infestation.

4. Conclusion

Blood glucose levels of grouper seeds (*Cromileptes altevalis*) which are kept in the Hatchery on concrete tanks range from 21-67 mg / dL. Grouper which is kept in the Hatchery with concrete tubs infested with ectoparasites which fall into the moderate to severe category. There is a high correlation level ($r = 1$) between blood glucose levels and ectoparasite infestations, the higher the blood glucose level, the higher the infestation of ectoparasite seeds of humpback grouper fish.

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