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Blood glucose and digestive tract andoparasite helminth infection of cantang grouper (*Epinephelus lanceolatus* x *Ephelus fuscoguttatus*) from traditional ponds in the Kampung Kerapu of Lamongan East Java

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Abstract. Cantang grouper *Epinephelus fuscoguttatus* x *Epinephelus lanceolatus* (*E. fuscoguttatus* x *E. lanceolatus*) is a hybrid fish between female tiger grouper and male kertang grouper, which has a high resistance to the environment and pathogen infection. The aim of this research was to find out the stress level (blood glucose levels) and helminth infections in the digestive tract of the cantang grouper (*E. fuscoguttatus* x *E. lanceolatus*) on a traditional pond in Kampung Kerapu Lamongan, East Java. This research is a survey research with 40 samples of Cantang grouper with purposive sampling. The results of the study showed that the Cantang groupers were reared in traditional ponds, positively infected by a single worms *Proisorhynchus*, with a prevalence of 24.4%. The lowest blood glucose levels was 0 mg/dL and the highest was 117 mg/dL. Infection was occurred in Cantang groupers that had blood glucose levels were below of normal, and above of normal with moderate infection degrees. The results also showed that there was no close correlation between blood glucose level of the Cantang grouper with intestinal helminth infections.

1. Introduction

One of the hybridized grouper fish that has been developed is aimed at obtaining superior seed quality is Cantang grouper *Epinephelus fuscoguttatus* x *Epinephelus lanceolatus* (*E. fuscoguttatus* x *E. lanceolatus*). Cantang grouper is a hybrid fish between female tiger grouper and male kertang grouper. The morphology characteristic of the Cantang grouper is similar to their parent species, but has better growth. In addition, the Cantang grouper has other advantages, namely high disease resistance, better tolerance to the environment and can be spread at high densities [1].

Besides having benefits, the potential of the Cantang grouper has increased production from year to year. Statistical data from [2] states that in 2015 grouper fish production reached 335.4 tons, in 2016 646.06 tons, and in 2017 it reached 742.96 tons.



Various kinds of obstacles in grouper aquaculture that need serious monitoring include water quality and disease attacks. This disease can infect both groupers that are kept in floating net cages (KJA) or in rearing ponds. Generally the obstacles that often arise in fish farming in ponds are only disease attacks and declining water quality Priagara *et al.* [3]. This is because the decreasing water quality will always be followed by increased in pathogenicity pathogens, so that they can infect the reared fish. Furthermore it is said that poor water quality management in the maintenance of fish in ponds, will cause the accumulation of organic material at the bottom of ponds. This will cause a decrease in water quality and support the bacteria growth that cause disease in fish. Besides that it will also cause a decrease in dissolved oxygen content and cause decay Isnawati *et al* [4]. Such conditions will cause fish to become stressed, which will be vulnerable to disease.

One indication that fish are stressed is an increase in blood sugar due to hormone secretion from the adrenal glands, increased respiratory rhythm, and the inflammatory response will be decreased by hormones from the adrenal glands. According to Nasichah *et al.* [5] when stressed fish will experience a primary and secondary response, where an increase in blood glucose is the primary response of a fish that experiences stress after a secondary response, namely an increase in the amount of stress hormones such as cortisol and catecholamines. Stressed fish will experience growth disturbance, productivity, homeostatic mechanisms in the body, and decreased endurance, making it vulnerable to illness, whether caused by parasites, bacteria, fungi or viruses Insivitawati *et al.* [6].

Parasitic infestations can occur due to unbalanced interactions between the environment, biota, and disease-causing agents Austin *et al.* [7]. Parasites are organisms that live on other organisms to take advantage of their host. Endoparasites have been found to infect the digestive tracts of cantang and tiger grouper are derived from groups of worms including *Anisakis* sp. with a prevalence of 50% Musyaffak *et al.* [8], *Anisakis physeteris* with a prevalence of 1%, *Neoechinorhynchus longnucleanus* with a prevalence of 3% Agustina *et al.* [9], *Lecithochirium magnoporum* with a prevalence of 5.9% and *Proisorhynchus luzonicus* with a prevalence of 40%, *Lecithochirium magnoporum* with a prevalence of 5.9% and *Proisorhynchus luzonicus* with a prevalence of 40% , *Camallanus carangis* with a prevalence of 62.9%, Rückert *et al.* [10].

Based on the background above, it is necessary to research the correlation between fish blood glucose levels and endoparasitic infections in the Cantang grouper (*E. fuscoguttatus* x *E. lanceolatus*). This will be used as scientific information about endoparasites that attack groupers. Information can be used as a grouper commodity selection by farmers so as to minimize losses due to endoparasitic infections.

2. Materials and Methods

2.1. Place and Time of Implementation

This research was conducted at the Laboratory of Aquaculture and Anatomy of the Faculty of Fisheries and Maritime Affairs, Airlangga University, Surabaya in August 2019. The method used in the study was a survey method by sampling at the location directly. Fish samples were taken from the grouper ponds in Labuhan Village, Lamongan Regency, East Java.

2.2. Preparation of Tools and Materials

The tools used in this study include; equipment for endoparasites: scissors, tweezers, scalpels, glass objects, glass covers, tweezers, hand tally counters, petridis, tissues, tablecloths, digital scales, ruler, microscopes and drops. While the equipment used for measuring blood glucose: a Glucose kit and 1 ml syringe. The materials used are 40-13 cm cantang grouper taken from traditional ponds in the Lamongan grouper village, glucose test script and 10% EDTA solution, as anticoagulant.

2.3 Sampling and Handling Techniques for Samples

40 samples were taken. Before examination of the parasite, the sample is measured in length with a ruler and weighed using pocket scales. After that, the fish are cut and surgically removed for inspection of parasitic worms.

2.4. Measurement of Blood Glucose Levels

Blood collection is done using a 1 ml syringe that has been moistened using an ethylene acid solution in which 10% tetracetate (EDTA) is 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 entered in the digital tool of the easy touch brand blood glucose test and then a number will come out showing the level of glucose in the blood. The results obtained are then recorded.

2.5. How to check for parasites in fish intestines

Examination of parasites in fish, parasites were carried out using techniques recommended by Fernando, *et al.* [11,12].

2.6. Intestinal Examination and Endoparasitic Infection

Examination of parasites in the digestive tract of the Cantang grouper (*E. fuscoguttatus* x *E. lanceolatus*) is carried out in a native way. Surgery is performed on a tray. The distinction is made by scissors from the anterior body to the lateral line then cut to the anal part of the fish. Examination of worms carried out on the intestine is placed in a petri dish filled with water then carried out scraping on the intestinal wall to remove the contents of feces in the intestine and placed on a glass object, covered with a glass cover. Then the liquid is stirred and observed under a microscope with a magnification of 40X and 100X. The data obtained are then identified and analyzed descriptively which will be presented in the form of figures and tables.

2.7. Identification

Endoparasites found were identified based on morphological characteristics. Parasite identification was carried out until the genus using identification books according to [13], Koesharyani *et al.* [14].

2.8. Data analysis

The data obtained were further analyzed descriptively to calculate the degree of infection based on the following formula.

$$\text{Infection degree} = \frac{\text{The number of parasites attacking fish}}{\text{The number of fish attacked by parasites}}$$

3. Result and discussion

3.1. Types and Amounts of Endoparasites

The results of the study showed that the Cantang grouper reared in traditional ponds, positively infected by the single worm *Posorhynchus longisaccatus*. The results of endoparasite examination of the grouper can be seen in Table 1.

Table 1. Results of endoparasite examination in cantang grouper

Types of Ectoparasites	The amount of infected fish
<i>Posorhynchus longisaccatus</i>	21
Negatif	19

The identification results show that the *Posorhynchus longisaccatus* found has an elongated body shape that is not blunt at both ends. The cuticle is covered by thorns and the body is widened in the ovary. The testes are located on the right side of the body and the genital pore is located in the posterior body. These characteristics are in accordance with the identification. The classifications of these worms are: Phylum: Platyhelminthes, Class: Trematode, Order: Plagiorchiida, Family: Bucccephalidae, Genus: *Posorhynchus* and Species: *Posorhynchus longisaccatus*. The testicles are round in a parallel position and are divided into two parts, namely the anterior testicle and the

posterior testicle. It has two elongated and tubular sperm glands. The genital pore is terminal in male worms, while the uterus is subterminal in female worms [13]. Pictures of the worms found are presented in Figure 1.



Figure 1. *Posorhynchus longisaccatus* found in cantang grouper, magnification 100X

3.2. Measurement of Blood Glucose Levels

The lowest blood glucose level of the fish was 0 mg / dL and the highest was 117 mg / dL, with the total number of cantang groupers infected with only 21 out of 40 samples. Infection occurs in both groupers that have below normal blood glucose levels, at normal and above normal levels, with normal to degrees of infection. The results of the measurement of blood glucose levels in the grouper (*E. fuscoguttatus* x *E. lanceolatus*) show that the blood glucose levels of groupers ranging from 0 mg / dL to 117 mg / dL, with a total infection of 0-5 individual worms per fish. This can be interpreted that groupers with blood glucose levels ranging from not detected up to 117 Mg / dL, are all infected with 0-5 individuals per fish, with the degree of infection from normal to slight. Blood glucose levels and total endoparasites and their infestation rates can be seen in Table 2.

Table 2. Results of measurement of blood glucose levels

Blood Glucose Levels (Mg/dL)	Total Endoparasites (individual)	Infection Degree
Undetected – 19	0 – 1	Normal – Slight
20 – 26 (Under Normal)	0 – 1	Normal – Slight
27 - 47 (Normal)	0 – 5	Normal – Slight
49 – 117 (Above Normal)	0 – 3	Normal – Slight

Table 2 showed that both groupers with glucose levels below normal, normal and above normal are infected with edoparasites as much as 0–5 individuals per fish, it can be interpreted that all groupers infected by worms endoparasite are in the normal-slight range. Water quality measurements are carried out before grouper fish sampling, so water quality measurements are carried out twice. The results of water quality measurements are supporting parameters during the study because environmental conditions are one of the stress factors in fish and the results obtained are water temperature 28 - 31 °C, DO (Dissolved Oxygen) 4 – 4,5 mg / L, pH 7-7,5, salinity 30-31 ppt and ammonia 1,2 mg / L. The Optimal water quality for groupers according to Mursitorini *et al.* [15] water temperatures in the KJA range from 27-31 °C. Salinity for grouper growth is around 30-33 ppt. The pH value of water ranged from 7.8 to 8.2 and DO in fish ponds in Kampung Labuhan Lamongan of

more than 5 ppm. Meanwhile, according to [16] explains that, the ideal water quality for the growth of grouper rats has an ideal water temperature ranging from 28-32 °C. The ideal salinity is 28-33 ppt, the ideal water pH ranges from 7,5 to 8,5, DO in ponds of 4 mg / L and ammonia ≤ 0.01 .

The results of the study showed that all of the cantang groupers, both those who were not stressed and those who were under stress, had all infected with *Posorhynchus longisaccatus* worms with an infection level between normal and slight. Some grouper fish that have blood glucose levels above normal can be interpreted that the fish are under stress. Stressed fish are shown with high glucose levels, due to the fact that the grouper abstain raised in the pond will occur physiological changes with an increase in glucose levels. The aborted grouper fish experienced a transition from hatchery to an enlarged pond, where environmental changes that occur can cause physiological changes in fish. This is due to fish cultivation carried out in ponds, leftover food, plankton and dead fish and other biota that are in waters that have died will settle and accumulate at the bottom of the pond Priagara *et al.* [3]. This condition will cause grouper to be stressed, besides that the condition is a medium that is suitable for the life of the parasite, so that there will be an increase in infection by the parasite [16]. The results of this study indicate that of the 19 fish grouper samples found to have high blood glucose levels and some even exceed normal. This can be interpreted that the fish experience stress. One of the physiological changes in fish when experiencing stress is an increase in blood glucose levels. High glucose levels are caused by the receipt of information causing stress by receptor organs, then the information is conveyed to the pituitary hypothalamus to secrete ACTH (Adrenocorticotrophic Hormone). This hormone will regulate cortisol secretion from interrenal cells. The hormone cortisol is located in the liver where gluconeogenesis occurs which results in an increase in blood glucose. Concurrently, chromaffine cells will secrete the catecholamine hormone. This hormone will suppress the secretion of the hormone insulin which serves to help supply glucose into cells, thereby causing glucose levels to enter the blood to increase [17].

Stressed Fish will experience a decrease in body defenses and cause susceptibility to parasitic infections. This is consistent with the opinion of [18] that, stress fish conditions have an impact on the decline of the immune system, inhibit growth, changes in swimming behavior, reproductive disorders and even death. Increased glucose levels can affect changes in fish behavior in the form of rapid operculum movement, fish take in air surface water, and fish become inactive [19]. The disease will attack fish if there are interactions between the host, pathogens, and the environment. This interaction causes stress on the fish, so the self defense mechanism weakens, and makes it easy for disease to enter the body and cause disease. The results of this study indicate that it is not in accordance with the above theoretical basis, in this study showed that 19 groupers (47,5%) who had blood glucose levels either below, normal (27 - 48 mg/dL) or above normal 0 - 117 mg/dL of all infected with *Posorhynchus longisaccatus* worm endoparasites with normal to mild degrees of infection. The worm *Posorhynchus longisaccatus* in its life cycle requires a host between the arthropods and crustaceans, so that in ponds where grouper fish samples are taken there is no such group. This causes the worm did not develop into an adult and did not have time to infect groupers as the definitive host. According to Brick *et al.* [20], stated that in addition to the presence of a host of high organic matter factors also support the development of the worm. This can be interpreted that the presence of these worms has not been in large numbers, so that even infected grouper fish found only an average of one individual worm in each one of the grouper fish and the degree of infection is also normal to mild. The measurement results of the pond water quality parameters where the groupers are kept, the water quality is still in a condition that can be tolerated by groupers, although the pH and ammonia content have suboptimal tendencies.

4. Conclusion

The conclusions that can be submitted from the results of this study are as follows: Provision Based on the results and discussion, the conclusion is endoparasite helminth infection of *Posorhynchus longisaccatus* were not always in stressed cantang grouper.

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