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Glucose Levels and Bacteria Identification in Cantang Groupers Distributed from Hatchery Bali

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ABSTRACT

The Cantang grouper is a hybrid species resulting from the crossbreeding of the female tiger grouper (*Epinephelus fuscoguttatus*) and the male kertang grouper (*Epinephelus lanceolatus*). Transporting fish using a closed transportation system can cause stress, which results in increased blood glucose levels in fish as a secondary physiological response. This study aims to analyze blood glucose levels and identify bacteria in cantang grouper (*Epinephelus fuscoguttatus lanceolatus*) distributed from hatcheries in Bali. The results showed that the average blood glucose level reached 102 mg/dL, higher than the normal range (27–56 mg/dL), indicating that the fish experienced stress due to bacterial infection and the transportation process. The cantang grouper observed had been infected with *Vibrio alginoliticus* bacteria with the lowest TVC value of 98 x 10^5 CFU/mL and the highest 239 x 105 CFU mL^{-1}. The relationship between blood glucose and TVC values is positively correlated (corellation = 1). The higher the blood glucose, the higher the TVC value in cantang grouper. Bacterial identification revealed the presence of *Vibrio alginolyticus*, a major pathogen causing vibriosis in marine fish. This study highlights the importance of improving transportation systems and implementing strict biosecurity to reduce stress and infection in cantang grouper.

INTRODUCTION

Fish comes from a cross (hybridization) of female tiger grouper (*Epinephelus fuscoguttatus*) and male kertang grouper (*Epinephelus lanceolatus*) which is one of the fishery commodities with the advantage of growing twice as fast as tiger grouper so that the production of cantang grouper increases every year (Ismi and Asih, 2017). The main problem faced in cantang grouper fish farming activities is the attack of pathogenic organisms that can cause major losses. One of the pathogenic

organisms that often attacks seawater fish is the Vibrio bacteria which causes vibriosis. Intensive fish farming increases the risk of infection due to factors such as poor water quality, high density, transportation that causes fish stress.

Stress is a number of physiological responses from the body that occur when animals try to maintain homeostasis in every demand imposed on them (Taqwa, 2008). Stress in fish is caused by poor environmental conditions (Susanto *et al.,* 2014). In stressful conditions, fish experience primary and secondary responses. The primary response is a change in conditions controlled by the Central Nervous System (CNS) by releasing stress hormones, namely cortisol and catecholamines (adrenaline and epinephrine) into the bloodstream through the endocrine system. Meanwhile, secondary responses occur due to the release of stress hormones that cause changes in blood and chemical tissues, such as increased glucose levels in the blood of fish (Syawal and Siregar, 2011).

Blood glucose serves as the primary energy source and essential substrate for cellular metabolism, particularly in brain function. For the brain to function continuously, glucose is needed continuously (Hastuti *et al.,* 2003). The blood volume in fish ranges from 1.5-3% of body weight. Normal blood glucose levels in fish contain 40-90 mg/dl, the blood glucose content is almost the same as blood glucose in humans, which is 70-110 mg/dl (Rahardjo *et al.,* 2011).

According to Rachmawati (2010), during stress, there is an increase in glucocorticoids which results in an increase in blood glucose levels to cope with high energy needs. If the blood glucose level of the fish is not normal, the fish's life will be disrupted and can even cause death. Measuring blood glucose levels can be used to diagnose fish experiencing stress simply and effectively for various stressors (Sulmartiwi *et al.,* 2013).

In the distribution of cantang grouper, the transportation method is a closed wet transportation system. Shipping uses styrofoam plastic bags, With limited oxygen during transportation, with a water-to-oxygen ratio of 1:3 (Kusyairi, 2013). A closed transportation system can expose fish to several stressors. Including catching, handling, population density, sudden changes in temperature, and physical trauma. This disorder causes fish to experience decreased immunity. Fish can also be attacked by disease if there is an interaction between the host, pathogens, and the environment, especially parasite and bacterial infestations (Suwandi *et al.,* 2019). The purpose of this study was to analyze blood glucose levels and TVC values of cantang grouper distributed from Bali Island.

LITERATURE REVIEW

Cantang Grouper Fish

The Cantang grouper is a type of grouper fish resulting from crossbreeding of a female tiger grouper (*Epinephelus fuscoguttatus*) with a male kertang grouper (*Epinephelus lanceolatus*) (Ismi and Asih, 2013). The hybridization aims to increase

growth rate by crossing the kertang grouper, which grows faster but is hard to breed, with the tiger grouper, which is easier to breed and develop. By combining these genetic traits, a hybrid fish can be produced that exhibits faster growth, has larvae that are easier to cultivate, is more resistant to disease, and is more tolerant of suboptimal environments and limited space (Rimmer and Glamuzina, 2019).

Blood Glucose

Increased blood glucose levels are a form of secondary physiological response. Increased or decreased blood glucose levels are often used as a benchmark to determine whether a fish is experiencing stress. In order to be called a biota experiencing increased glucose levels or not, it can be seen from the optimal value of blood glucose levels. Each biota has a different optimal blood glucose level, therefore if it enters the optimal range, a biota can be said to be healthy. The optimal glucose level for grouper fish is in the range of 27 - 56 mg / dL (Mahasri *et al.,* 2022).

Identification of Vibrio Bacteria

Vibrio is a genus of gram-negative, single-celled, short, curved rod-shaped bacteria (comma), measuring 1.4–5.0 μm long and 0.3–1.3 μm wide, motile and having polar flagella and typically found in seawater. Vibrio is facultative anaerobic, meaning it can live with or without oxygen. All members of the Vibrio genus are motile (moving) and have polar flagella with protective sheaths (Michel, 1986). There are 3 important types of Vibrio bacteria known to attack grouper fish, namely *Vibrio alginolyticus* and *Vibrio parahaemolyticus*, and Vibrio harveyi. These three bacteria and several other strains have been isolated from sick grouper fish (Ali, 2005).

METHOD

Time and Place

This research was conducted in June 2024. The samples used were 30 cantang grouper fish measuring 10 cm in length and weighing 9 grams. Sampling of 30 cantang grouper fish was carried out in it is hoped that it can represent the existing fish population in Bali . The research was conducted at the Anatomy and Cultivation Laboratory, Faculty of Fisheries and Marine Affairs, Airlangga University.

Blood Glucose Measurement

Fish blood glucose was measured using a modified method from (Tang *et al.,* 2018), first 0.1 ml of fish blood was taken using a syringe. Next, blood glucose was measured using the Easytouch GCHb blood glucose meter by dripping blood on a special test strip for blood glucose meter that has been installed on the device. The results will be visible on the screen. The value printed on the device represents the fish's blood sugar in mg/dL.

Identification of Bacteria

Isolating bacteria is done in a laminar air flow with the source of the isolate being the surface of the fish skin and kidneys. Isolation is done by mucus in the wound on the surface of the fish skin using a sterile ose needle then scratched into selective agar TCBS media in a zigzag manner. The results of bacterial isolation were incubated at a temperature of 26-30ºC for 24-48 hours. Bacterial purification was carried out by taking bacteria found in the media that had uniform colony shapes, colors, and sizes and then inoculating them repeatedly into the bacterial growth media.

Observation of bacterial colony morphology is carried out after obtaining pure cultures. Observation of bacterial colony morphology by looking at the color, shape, edge, surface of the colony (elevation) directly from the bacterial growth medium. Gram staining is one of the most important bacterial identification techniques in determining the type of bacteria. Biochemical tests are a method used to determine the chemical reactions that occur in the body of microorganisms. The results of biochemical tests can be used to identify a microorganism. According to (Buller, 2004) biochemical tests used in bacterial identification activities include: catalase, oxidase, O/F, TSIA, motility, indole and MR-VP tests.

Data analysis

Data analysis in this study was conducted using descriptive analysis and presented in the form of images and tables. The data obtained will be compared with the bacterial identification book "*Cowan and Steel's Manual for the Identification of Medical Bacteria*"

RESULT AND DISCUSSION

RESULT

Blood Glucose Observation

The results of the blood glucose observations of the cantang grouper fish that have been conducted can be seen in Table 1.

Table 1. Blood Glucose of Cantang Grouper Fish

Fish observed	Blood Glucose (mg/dL)	Category	The presence of Vibrio bacteria	TVC value CFU/mL x 10^5
1	61	*	+	98
2	93	*	+	156
3	86	*	+	112
4	90	*	+	119
5	75	*	+	102
6	93	*	+	156
7	123	*	+	217
8	154	*	+	232

Fish observed	Blood Glucose (mg/dL)	Category	The presence of Vibrio bacteria	TVC value CFU/mL x 10^5
9	94	*	+	186
10	113	*	+	201
11	93	*	+	173
12	87	*	+	112
13	95	*	+	193
14	73	*	+	101
15	87	*	+	114
16	119	*	+	204
17	137	*	+	226
18	121	*	+	212
19	97	*	+	194
20	156	*	+	239
21	98	*	+	198
22	135	*	+	223
23	117	*	+	203
24	123	*	+	219
25	72	*	+	101
26	83	*	+	109
27	79	*	+	103
28	94	*	+	191
29	131	*	+	221
30	82	*	+	103
Average	102	abnormal (stress)	Positive bacteria (+)	167 CFU/mL

Description: * (abnormal), ** (normal), + (positive for bacterial infection), - (not infected with bacteria)

Table 1 shows that the glucose value of grouper fish after being distributed from Bali showed above normal values with the lowest glucose value being 61 mg/dL and the highest being 156 mg/dL with an average blood glucose of 30 fish samples of 102 mg/dL. The blood glucose levels of cantang grouper fish increased drastically from normal blood glucose levels, which were in the range of 27–56 mg/dL (Mahasri *et al.,* 2022). The cantang grouper fish observed had been infected with *Vibrio alginoliticus* bacteria with the lowest TVC value being 98 x CFU mL^{-1} and the highest being 239 x 105 CFU mL^{-1}.

The relationship between blood glucose and TVC value is positively correlated. The higher the blood glucose, the higher the TVC value in cantang grouper fish (corellation = 1). The results of observations for grouper fish infected with bacteria showed high blood glucose values. This is thought to be due to

bacterial infection that releases toxins that cause host stress, thus increasing blood glucose levels in the blood. When fish experience stress, such as due to bacterial

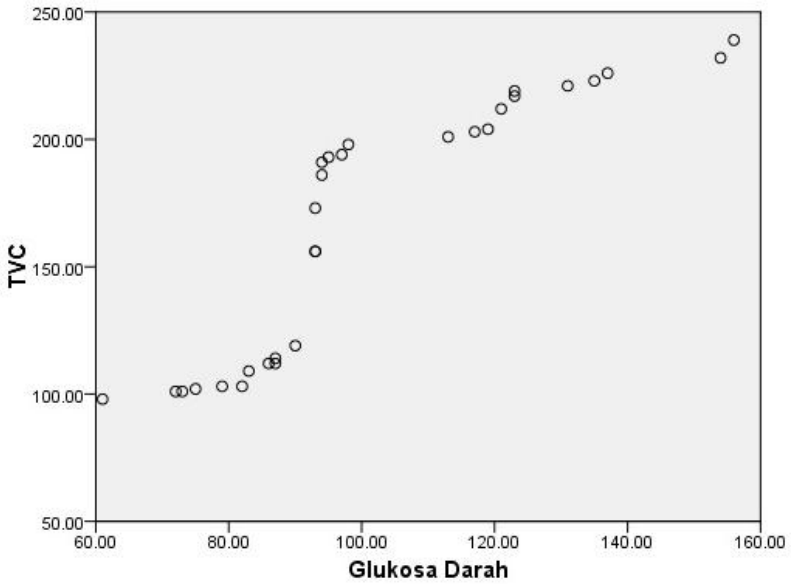

Figure 1. Correlation of blood glucose with TVC value of Cantang grouper fish

infections, environmental changes, or other factors, the fish's endocrine system responds by releasing cortisol from the adrenal glands. Cortisol is known as the stress hormone that helps the body adapt to stressful conditions. Cortisol can also increase the release of glucose from glycogen stores in the liver into the bloodstream. As a result, the fish's blood glucose levels will rise to support the increased energy needs of the body during stress. According to Minaka *et al.,* (2012) blood glucose can be one indicator of stress in fish. This condition will cause host stress and increase blood glucose levels and cause young fish to be infected with bacteria as seen from the increased TVC value.

Identification of Bacteria

The results of the identification of the cantang grouper fish can be seen in Table 2.

Table 2. Biochemical Test of Bacteria

Characteristics	*Vibrio alginolyticus*
Oxidase	+
Catalase	+
O/F	F
TSIA – H2S	-
Motility	+
Indole	+
MR	+
VP	+

Bacterial characterization relies on morphological and biochemical tests. Biochemical tests conducted in this study include oxidase, catalase, O/F, TSIA, Motility, Indole, MR and VP tests. From the results of morphological observations and biochemical tests compared with Cowan and Steel's book, "*Manual for the Identification of Medical Bacteria", it was found that the characteristics match those of Vibrio alginolyticus* bacteria.

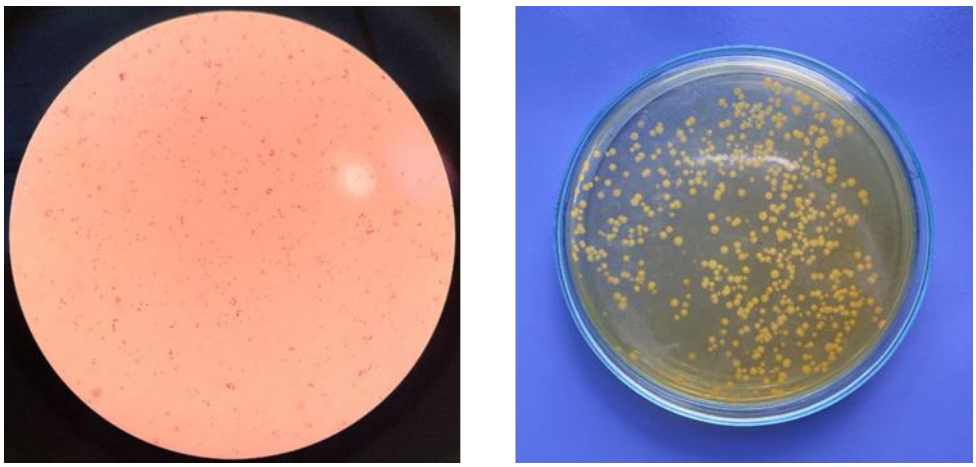

Figure 2. *Vibrio alginolyticus* bacteria

In this study, *V. alginolyticus* bacteria were cultured on TCBS media. The results showed that *V. alginolyticus* bacteria were able to change the color of the TCBS media from green to yellow because the bacteria can ferment sucrose in the media. This color change was clearly observable in Figure 2.

Water Quality

The supporting parameters in this study were the water quality of the cantang grouper fish maintenance in the Bali hatchery . The water quality parameters observed during the study included temperature, dissolved oxygen (DO), salinity, and pH. The values of the water quality parameters during maintenance can be seen in Table 3.

Table 3. Maintenance Water Quality

Treatment	Temperature	DO	pH	Salinity
K-	30.00	5.07	7.5	31
K+	30.25	5.07	7.6	31
P1	29.75	5.06	7.5	31
P2	29.50	5.03	7.5	31

Based on the results of water quality parameter measurements that have been carried out (Table 3), it can be stated that the water quality for maintaining cantang grouper fish in the Bali hatchery is within the normal range according to SNI 8036.2 (2014).

DISCUSSION

Blood glucose levels of Bali grouper from 30 samples showed values ranging from 61-15 6 mg / dL with an average of 102 mg / dL. Fish experience changes in physiological responses consisting of primary responses and secondary responses. The primary response occurs with an increase in the number of hormones such as catecholamines and cortisol, while the secondary response that occurs is an increase in blood glucose (Barton, 2002). This is thought to be because the higher blood glucose levels indicate the state of the fish in a state of stress due to shocks during transportation which causes increased movement activity and oxygen consumption and higher fish metabolism (Wahyu, 2015).

One of the characteristics of stressed fish is increased glucose levels in blood plasma. The presence of a stress response will stimulate the hypothalamus to release corticotrophin releasing factor (CRF), which will stimulate the anterior pituitary gland to release adrenocorticotropic hormone (ACTH). ACTH then stimulates interrenal cells (adrenal medulla) to produce cortisol and catecholamine hormones, such as epinephrine. These hormones play a role in the gluconeogenesis process which will deposit glycogen reserves in the liver and muscles to increase blood glucose levels (Hastuti, 2004).

Increased glucocorticoids due to stress affect the increase in blood glucose levels. This serves to overcome high energy needs during stress (Rachmawati et al., 2010). The energy needs of glucose to deal with stress can be met if glucose in the blood can immediately enter the cells. Glucose that has entered the cells will be immediately used in metabolism to meet the physiological needs of the body and energy. The supply of glucose that has been met will stimulate the process of glycogenesis and lypogenesis. In addition to increased blood glucose can cause stress, bacterial infections also affect the stress levels of fish. In this study, cantang grouper fish were infected with *Vibrio alginolitycus bacteria .*

Vibrio alginolyticus bacteria in the identification process including Gram negative bacteria, morphological form of bacillus cells (rods), brownish yellow colony color, spread, grooved edges and flat elevation (same height as medium), and biochemical tests are oxidase and catalase positive, fermentative, motile, indole positive, MR and VP positive. *Vibrio alginolitycus bacteria* are very common in brackish and marine waters. According to Desrina *et al.,* (2006) of several species of bacteria that are often found in sick grouper fish are *V. alginolitycus bacteria*.

CONCLUSION

Cantang grouper (*Epinephelus fuscoguttatus lanceolatus*) distributed from hatcheries in Bali experienced stress due to the closed transportation system, as indicated by an average blood glucose level of 102 mg/dL, above the normal range (27–56 mg/dL). Increased blood glucose levels occur as a physiological response to stress caused by handling, high density, temperature changes, and physical trauma

during transportation and bacterial infection. The observed cantang grouper fish were infected with *Vibrio alginoliticus* bacteria with the lowest TVC value of 98 x 10^5 CFU mL^{-1} and the highest of 239 x 10^5 CFU mL^{-1}. The relationship between blood glucose h and TVC value is positively correlated. The higher the blood glucose h , the higher the TVC value in cantang grouper fish. Identification of bacteria showed the presence of *Vibrio alginolyticus*, the main pathogen causing vibriosis which is often found in saltwater fish. It is advisable to manage water quality, use probiotics, or implement pre-transport treatments to prevent bacterial infections that cause fish stress. The results of this study emphasize the importance of implementing better transportation systems to reduce stress on fish as well as implementing strict biosecurity to prevent bacterial infections during the distribution process.

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