



# PHYSIOLOGICAL RESPONSE OF CANTANG GROUPER AS ZEYLANICOBDELLA CONTROL WITH PAPAYA LEAF EXTRACT AS AN ANTIPARASITIC AGENT

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## Abstrak

Permintaan pasar terhadap ikan kerapu cantang yang terus meningkat menyebabkan intensifikasi budidaya tidak dapat dihindarkan. Salah satu kendala utama dalam upaya peningkatan produksi ikan kerapu adalah serangan penyakit. Alternatif bahan obat yang dapat digunakan dalam pengendalian penyakit yaitu dengan menggunakan daun pepaya lokal dan daun pepaya jepang. Tujuan dari penelitian ini adalah untuk menganalisis pengaruh konsentrasi dan lama waktu perendaman ekstrak daun pepaya sebagai antiparasit terhadap kadar glukosa darah dan tingkat konsumsi oksigen selama proses pengendalian *Zeylanicobdella* pada ikan kerapu cantang. Metode yang digunakan adalah metode eksperimental dengan rancangan acak lengkap faktorial 6 perlakuan dan 3 ulangan. Hasil dari penelitian ini yaitu diperoleh hasil rata-rata kadar glukosa yang diberi perlakuan ekstrak daun pepaya jenis berbeda selama dua jam menunjukkan bahwa pada faktor A dan B terdapat pengaruh dan interaksi yang sangat nyata ( $p < 0,01$ ) terhadap kadar glukosa darah, serta interaksi yang nyata ( $p < 0,05$ ) antara faktor A dan B terhadap tingkat konsumsi oksigen. Berdasarkan hasil penelitian dapat disimpulkan bahwa terdapat interaksi yang sangat nyata antara lama waktu perendaman dan konsentrasi ekstrak daun pepaya lokal dan pepaya jepang sebagai bahan antiparasit terhadap peningkatan kadar glukosa darah dan terdapat interaksi yang nyata terhadap tingkat konsumsi oksigen selama proses pengendalian *Zeylanicobdella* pada ikan kerapu cantang

**Kata Kunci:** Daun pepaya jepang, Daun pepaya lokal, Kerapu cantang, *Zeylanicobdella*

## Abstract

Efforts to increase grouper production are disease attacks. Alternative medicinal ingredients that can be used in disease control are using local papaya leaf and Japanese papaya leaf. The purpose of this study was to analyze the effect of concentration and duration of dipping in papaya leaf extract as an antiparasitic on blood glucose levels and oxygen consumption levels during the *Zeylanicobdella* control process in cantang grouper. The method used is an experimental method with a completely randomized factorial design with 6 treatments and 3 replications. The results of this study are the average results of glucose levels treated with different types of papaya leaf extract for two hours indicate that factors A and B have a very significant effect and interaction ( $p < 0.01$ ) on blood glucose levels, and significant interaction ( $p < 0.05$ ) between factors A and B on the level of oxygen consumption. Based on the results of the study, it can be concluded that there is a very significant interaction between the length of soaking time and the concentration of local papaya leaf extract and Japanese papaya as antiparasitic ingredients to increase blood glucose levels and there is a significant interaction with the level of oxygen consumption during the *Zeylanicobdella* control process in cantang grouper.

**Keywords:** Japanese papaya leaf, Local papaya leaf, Cantang grouper, *Zeylanicobdella*

## 1. INTRODUCTION

Grouper is one of the mainstay commodities of marine aquaculture in

Indonesia. Grouper production in East Java according to the Ministry of Maritime Affairs and Fisheries (2020) in 2019 - 2020 is 1196

tons and 1606 tons, respectively. Market demand for fishery products continues to increase, causing the implementation of aquaculture intensification to be unavoidable. One of the main obstacles in efforts to increase grouper production is disease. According to Arwin *et al.* (2016) diseases in fish include infectious and non-infectious diseases. Infectious diseases are diseases caused by pathogens such as viruses, bacteria, fungi, and parasites. Most of the types of parasites that infest groupers and cause significant losses are ectoparasites.

One type of ectoparasite that has been reported to attack cultured marine fish since 2015 is the Hirudinea sea leech, which comes from the species *Z. arugamensis*. The existence of these leeches is still a major obstacle for fish farmers. Based on the research of Shah *et al.* (2019) obtained data that *Zeylanicobdella* attacked grouper with 100% mortality in the hatchery of the University of Malaysia, Sabah. The prevalence of *Z. arugamensis* infesting cantang grouper is based on the research of Mahasri *et al.* (2020) which is 100% in the ground pool. In addition, based on the research of Mahardika *et al.* (2018) conducted at the Teluk Kaping KJA, North Bali, obtained data that *Zeylanicobdella* was always found every month during the five months of the study from February - June 2018 and decreased the population by around 44.9% - 47.53%.

Alternative medicinal ingredients that can be used in disease control is by using papaya leaf. Papaya leaf is a medicinal plant that is easily obtained by fish cultivators including the local papaya (*Carica papaya* L) and Japanese papaya (*Cnidioscolus aconitifolius*), both types of these plants are very easy to grow, especially in the highland areas with cool climates. Local papaya leaf, local papaya (*Carica papaya* L) and Japanese papaya (*Cnidioscolus aconitifolius*) contain chemical compounds that are antiparasitic. Local papaya leaf contain alkaloids karpain, pseudokarpain, vitamins C and E, choline, and carposid. Based on the statement of

A'yun and Laily (2015) that the content of bioactive substances in 100 grams of local papaya leaf contains flavonoids by 14% and saponins by 30%. The tannin content in papaya leaf is 14% (Akachukwu, 2014). Meanwhile, according to Obichi *et al.* (2015) the content of bioactive substances in Japanese papaya leaf includes tannins of 5.72%.

Control of *Zeylanicobdella* by giving different concentrations of local papaya leaf extract (*Carica papaya* L) and Japanese papaya (*Cnidioscolus aconitifolius*) can affect the stress level of cantang grouper, because the addition of papaya leaf extract will cause water as a living medium for fish to experience changes both physically and mentally. chemical. During the dipping time of fish, stress will have an impact on blood glucose levels, levels of oxygen consumption and levels of the hormone cortisol. Blood glucose levels increase due to hormone secretion from the adrenal glands which is characterized by increased respiratory rhythm and will increase the hormone cortisol. The level of oxygen consumption increases because fish carry out adaptation activities so that the oxygen needed also increases (Djauhari *et al.*, 2019). Therefore, it is necessary to conduct research on the concentration and duration of dipping of local papaya leaf extract and Japanese papaya leaf extract as an antiparasitic to the physiological response to suppress *Zeylanicobdella* infestation in grouper cantang.

## 2. RESEARCH METHODS

### 2.1 Time and Place of Research Implementation

This research was conducted in January – April 2022. The treatment of dipping of cantang grouper with different types of papaya leaf extract, measurement of blood glucose levels, oxygen consumption levels, cortisol hormone levels and tachiventilation were carried out at the Anatomy and

Aquaculture Laboratory. Different types of papaya leaf extract were made at the Laboratory of Chemistry and Analysis, Faculty of Fisheries and Marine Affairs, Airlangga University, Surabaya

determined as LD<sub>50</sub> because at this concentration the grouper could not survive, so the concentration of papaya leaf extract to be used for treatment was less than a concentration of 1%.

## 2.2 Preliminary Research

The preliminary study used 20 cantang groupers with five treatments and four replications, namely P0 (control), P1 local papaya leaf extract (*Carica papaya* L) 0.5% concentration, P2 local papaya leaf extract (*Carica papaya* L) concentration 1 %, P3 Japanese papaya leaf extract (*Cnidoscopus aconitifolius*) with a concentration of 0.5%, and P4 Japanese papaya leaf extract (*Cnidoscopus aconitifolius*) with a concentration of 1%. The reference concentration used in the preliminary study came from a previous study, namely Rochmad's (2021) study which used papaya leaf juice (*Carica papaya* L.) to control *Zeylanicobdella* in vivo with concentrations of 0.1%, 0.3% and 0.5%. and the effective concentration that made *Zeylanicobdella* released entirely from the host was at a concentration of 0.5% with a treatment time of two hours. The reference concentration used in the preliminary research of papaya leaf extract was carried out with the smallest concentration first, this is because there has been no previous research on papaya leaf extract that determines the optimal concentration. Preliminary research on papaya leaf extract used concentrations of 0.5% and 1%. The results of the preliminary study showed that the concentration where *Zeylanicobdella* infesting cantang grouper had a mortality of 50% occurred in the treatment of Japanese papaya leaf extract (*Cnidoscopus aconitifolius*) with a concentration of 0.5%, so that the concentration of 0.5% was determined as LC<sub>50</sub>. In addition, fish cannot survive for two hours or die at a concentration of 1% and fish can survive for two hours at a concentration of less than 1%. The concentration of 1% was

## 2.3 Research Design

This study used a completely randomized design (CRD) with a factorial pattern consisting of factor A and factor B with 6 treatments and 3 replications. Factor A is treatment with different concentrations of papaya leaf extract, namely A1 (local papaya leaf extract concentration 0.25%), A2 (local papaya leaf extract concentration 0.5%), A3 (local papaya leaf extract concentration 0.75%) , A4 (Japanese papaya leaf extract concentration 0.25%), A5 (Japanese papaya leaf extract 0.5% concentration), and A6 (Japanese papaya leaf extract concentration 0.75%) and factor B is a different dipping time, namely B1 (0 minute), B2 (60 minute) and B3 (120 minute).

## 2.4 Research Preparation

Aquarium is cleaned first, then the aquarium is filled with water and a chlorine solution is added. The aquarium which has been filled with water and chlorine solution is left for 24 hours, then washed with fresh water until it is free from the smell of chlorine and dried. The arrangement of a series of maintenance containers includes installing a blower, aeration hose, aeration stones and placing the aquarium on a rack.

## 2.5 Sampling

Preparation of cantang grouper began with sampling at the location of Klatakan Village, Panarukan District, Situbondo Regency. 20-25 cm Cantang groupers infested with the *Zeylanicobdella* parasite were then transported openly using a drum filled with 100 liters of seawater and given pure oxygen and given ice cubes until the



**Figure 1.** Cantang groupers infested with the *Zeylanicobdella* parasite on pectoral fin

## 2.6 Blood glucose level measurement

Sampling of cantang grouper fish was taken from each aquarium, so that a total of six samples were obtained from each treatment. Cantang grouper blood was taken using a 1 ml syringe filled with 10% EDTA. The blood of the cantang grouper is taken from the caudalis vein, which is a blood vessel located right on the ventral part of the vertebrae (backbone) (Hidayaturrahmah, 2015). The blood that has been taken is inserted at the end of the test script that has been entered in the digital glucose test tool. The results of glucose levels will be displayed on the device screen in the form of numbers with units of mg/dL, then the results are recorded. Blood glucose measurements were carried out at the 0, 60 and 120 minutes of treatment.

## 2.7 Calculation of Oxygen Consumption level

Sampling of cantang grouper fish was taken from each aquarium, so that a total of six samples were obtained from each treatment. The cantang grouper sample was

then used for calculating the level of oxygen consumption. Before calculating the level of oxygen consumption, the weight of the cantang grouper was first measured by weighing it using an analytical balance. Blood glucose measurements were carried out at the 0, 60 and 120 minutes of treatment. Calculation of the level of oxygen consumption using the formula according to Djauhari *et al* (2019) as follows:

$$TKO = \frac{V \times (DOT_0 - DOT_t)}{W \times t}$$

Description:

TKO : Oxygen consumption level (mg O<sub>2</sub>/g/hour)

V : Volume of water in the container (L)

DOT<sub>0</sub> : Dissolved oxygen concentration at the beginning of observation (mg/L)

DOT<sub>t</sub> : Concentration of dissolved oxygen at time t (mg/L)

W : weight of test fish (g)

t : Observation period (hours)

## 2.7 Data analysis

Data analysis in this study used IBM SPSS (Statistical Package for the Social Sciences) Statistics Version 24. The statistical test used was Analyze of Variance (ANOVA) completely randomized design (CRD) factorial pattern to determine the effect of the treatment given. If there is a significant difference, it will be continued with Duncan's Multiple Range Test (Duncan's Multiple Range Test) to find out the best treatment.

## 3. RESULTS AND DISCUSSION

### 3.1 Blood glucose level

Based on the research that has been done, the results of the average blood glucose levels in cantang grouper that were treated with different types of papaya leaf extract for two hours showed that factors A and B had a



very significant effect ( $p < 0.01$ ) and there was an interaction between which is very significant between factors A and B on blood

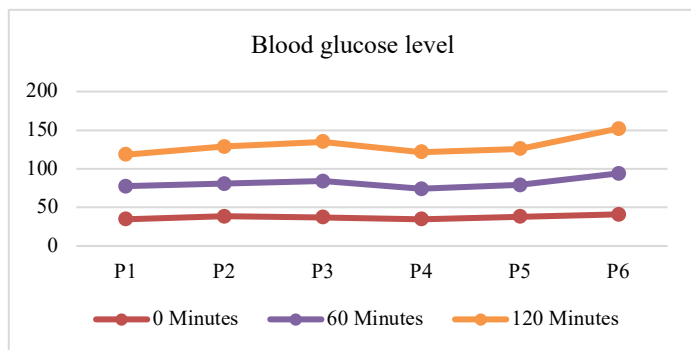
will affect the increase in blood glucose levels, this is due to the addition of high concentration of papaya leaf extract in the treatment water media will stimulate changes by the receptor organs that will secrete catecholamine hormones. This hormone will activate the enzymes involved in the catabolism of liver and muscle glycogen stores and suppress the secretion of the hormone insulin, so that blood glucose increases as well. The lowest glucose levels were in A1 and A4 with an dipping time of 0 minutes, this was due to the absence of the addition of papaya leaf extract so that it did not increase cortisol levels and did not reduce insulin action in the blood. The availability of insulin which mobilizes blood glucose into normal moving cells will result in blood glucose in normal conditions (Hastuti *et al*, 2003).

Dipping time	Blood glucose level (mg/dL) ± SD		
	B1	B2	B3
A1	34,6 <sup>h</sup> ± 1,52	42,6 <sup>d</sup> ± 1,52	45,6 <sup>c</sup> ± 1,52
A2	38,3 <sup>fg</sup> ± 1,52	42,3 <sup>d</sup> ± 1,52	48,3 <sup>c</sup> ± 1,52
A3	37 <sup>gh</sup> ± 2,01	47 <sup>c</sup> ± 2,01	51 <sup>b</sup> ± 2,01
A4	34,6 <sup>c</sup> ± 1,52	39,6 <sup>ef</sup> ± 2,07	47,6 <sup>c</sup> ± 2,07
A5	38 <sup>fg</sup> ± 1,01	41 <sup>dc</sup> ± 1,01	47 <sup>c</sup> ± 1,01
A6	41 <sup>de</sup> ± 1,01	53 <sup>b</sup> ± 1,01	58 <sup>a</sup> ± 1,01

glucose levels of cantang grouper ( $p < 0.01$ ), the following data can be explained in Table 1.

**Table 1.** Average Blood Glucose Levels of Cantang Grouper During Treatment

Different superscripts in the same column and row show very significant different effects ( $p < 0.01$ )



**Figure 2.** Graph of average blood glucose level

Table 1 shows that the highest blood glucose levels occurred in treatment A6 (Japanese papaya extract concentration of 0.75%) with an dipping time of 120 minutes, namely 58 mg/dL. The lowest glucose levels were in treatment A1 (local papaya extract concentration 0.25%) with an dipping time of 0 minutes, namely 34.6 mg/dL and in treatment A4 (Japanese papaya extract 0.25% concentration) with an dipping time of 0 minutes, namely 34.6 mg/dL. The higher the concentration of papaya leaf extract used, it

Blood glucose levels increased at doses of 0.25%, 0.5% and 0.75%, this was related to the mobilization of energy storage under stress conditions as a fuel source for anaerobic metabolism resulting in the production and accumulation of lactate due to the presence of one or more flavonoid compounds and tannins contained in local papaya leaf extract (*Carica papaya* L) and Japanese papaya leaf extract (*Cnidioscolus aconitifolius*) (Haryoto and Devi, 2018). Based on the results of the analysis of blood glucose levels, papaya leaf extract at minute 0 approached the optimal blood glucose level of cantang grouper. This condition indicates that at minute 0 the fish have not utilized enough blood glucose in the blood as an energy source, so that the blood glucose concentration will decrease to a normal point. The increase in glucose concentration is related to the mobilization of energy stores under stress conditions as a fuel source for anaerobic metabolism resulting in the production and accumulation of lactate (Arifin *et al.*, 2014).

### 3.2 Oxygen consumption level

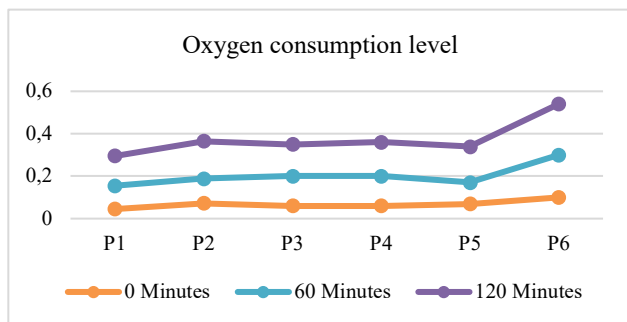


Based on the research that has been done, the results of the average level of oxygen consumption in cantang grouper that were treated with different types of papaya leaf extract for two hours showed that factors A and B had a very significant effect ( $p < 0.01$ ) and there was an interaction between The significant difference between factors A and B on the oxygen consumption level of cantang grouper ( $p < 0.05$ ), the following data can be explained in Table 2.

**Table 2.** Average oxygen consumption level of Cantang Grouper During Treatment

Dipping time	Oxygen consumption level (mgO <sub>2</sub> /g/hour) ± SD		
	B1	B2	B3
A1	0,05 <sup>i</sup> ± 0,01	0,11 <sup>ef</sup> ± 0,02	0,14 <sup>dc</sup> ± 0,02
A2	0,073 <sup>ghi</sup> ± 0,025	0,116 <sup>ef</sup> ± 0,03	0,176 <sup>bc</sup> ± 0,03
A3	0,08 <sup>fgh</sup> ± 0,015	0,196 <sup>b</sup> ± 0,015	0,23 <sup>a</sup> ± 0,015
A4	0,06 <sup>hi</sup> ± 0,02	0,14 <sup>de</sup> ± 0,02	0,16 <sup>cd</sup> ± 0,02
A5	0,07 <sup>ghi</sup> ± 0,01	0,1 <sup>fg</sup> ± 0,01	0,17 <sup>bcd</sup> ± 0,01
A6	0,1 <sup>fg</sup> ± 0,02	0,2 <sup>b</sup> ± 0,02	0,24 <sup>a</sup> ± 0,02

Different superscripts in the same column and row show very significant different effects ( $p < 0.01$ )



**Figure 3.** Graph of average oxygen consumption level

Table 2 shows that the highest level of oxygen consumption occurred in treatment A6 (Japanese papaya extract concentration of 0.75%) with an dipping time of 120 minutes, namely 0.24 mgO<sub>2</sub>/g/hour. The lowest glucose levels were in treatment A1 (local papaya extract concentration 0.25%) with an dipping time of 0 minutes, namely 0.05 mgO<sub>2</sub>/g/hour and in treatment A4 (Japanese papaya extract 0.25% concentration) with an dipping time of 0 minutes is 0.06 mgO<sub>2</sub>/g/hour. The higher the concentration of papaya leaf extract used, it will affect the

increase in the level of oxygen consumption. Based on this, it shows that fish make adaptations to adapt to environmental conditions so that fish do a lot of movement and require higher oxygen, causing DO to decrease after being treated with papaya leaf extract. As a result of high oxygen consumption, it triggers an increase in the respiration process so that it has an impact on increasing temperature because along with the increase in the respiration process (removal of carbon dioxide from the body) will affect the environmental temperature which is increasing as well. The conditions of the water media that are increasingly cloudy also affect the availability of dissolved oxygen and oxygen consumption by fish. This is supported by Putra's (2015) statement that stressed fish need a lot of energy to adapt to environmental conditions, increased oxygen consumption and carbon dioxide expenditure indicate an increased respiration process. Increasing the concentration of carbon dioxide or hydrogen ions will cause a decrease in pH. It is also supported by the statement of Djauhari *et al* (2019) which states that at a temperature of 21-27 °C there tends to be an increase in metabolism so that respiration increases the excretion of ammonia.

The increase in the level of oxygen consumption indicates the metabolic rate of the fish is also increasing. The level of oxygen consumption increased at the time of dipping in the 60th minute and 120th minute. This is presumably because the fish are stressed, so it takes a lot of energy to adjust to the aquarium conditions. Increased levels of oxygen consumption and carbon dioxide expenditure indicate an increased respiration process. This increased respiration process will increase the metabolic rate of fish, especially basal metabolism. According to Halver and Ronald (2002), basal metabolism includes the process of respiration, blood circulation, intestinal peristalsis.

#### 4. CONCLUSIONS AND SUGGESTIONS



Based on the results of the research that has been done, it can be concluded several things as follows:

1. Concentration of local papaya leaf extract (*Carica papaya* L) and Japanese papaya (*Cnidioscolus aconitifolius*) as antiparasitic agents has a very significant effect on blood glucose levels and oxygen consumption levels during the *Zeylanicobdella* control process in grouper cantang
2. The duration of dipping of local papaya leaf extract (*Carica papaya* L) and Japanese papaya (*Cnidioscolus aconitifolius*) as antiparasitic ingredients has a very significant effect on blood glucose levels and oxygen consumption levels during the *Zeylanicobdella* control process in cantang grouper.
3. There is a very significant interaction between the dipping time and the concentration of local papaya leaf extract (*Carica papaya* L) and Japanese papaya (*Cnidioscolus aconitifolius*) as antiparasitic agents on increasing blood glucose levels and there is a significant interaction with the level of oxygen consumption during the *Zeylanicobdella* control process. on cantang grouper

Based on the results of the research that has been carried out, the authors suggest to do the recovery of grouper cantang that has been given extracts of local papaya leaf (*Carica papaya* L) and Japanese papaya (*Cnidioscolus aconitifolius*) so that blood glucose levels and oxygen consumption levels return to normal.

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