



THE EFFECT OF TRANSPORTATION ON BLOOD GLYCOSE LEVELS AND ECTOPARASITE INVESTMENT IN PUNTEN CARP (*Cyprinus carpio*)

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Abstrak

Ikan mas merupakan salah satu sumber protein hewani yang banyak diminati. Untuk memenuhi kebutuhan permintaan ikan mas, distribusi hasil budidaya perlu dilakukan yaitu dengan sistem transportasi. Teknik transportasi yang kurang baik akan menyebabkan kondisi ikan memburuk, sehingga meningkatkan stres dan tingkat kematian ikan saat perjalanan. Stres dapat menyebabkan daya tahan ikan menjadi lemah, sehingga parasit mudah masuk ke dalam tubuh ikan. Tujuan penelitian ini adalah untuk mengetahui adanya pengaruh transportasi terhadap kadar glukosa darah dan infestasi ektoparasit pada ikan mas Punten. Metode yang digunakan dalam penelitian adalah metode eksperimental dengan membandingkan nilai kadar glukosa darah dan infestasi parasit pada ikan sebelum proses transportasi dan setelah proses transportasi. Hasil analisa kualitas air sebelum proses transportasi dengan suhu 270C, pH 7,6, Oksigen Terlarut 5 mg/l, ammonia 0,05 mg/l, dan setelah proses transportasi dengan suhu 280C, pH 7, Oksigen Terlarut 5 mg/l, ammonia 0,1 mg/l masih dalam batas normal. Pada perlakuan transportasi ikan Mas Punten dapat meningkatkan kadar glukosa darah ikan yang signifikan dengan nilai sebelum dilakukan transportasi sebesar 31,54 mg/dL dan sesudah dilakukan transportasi sebesar 51,41 mg/dL. Peningkatan lestasi ektoparasit yang tidak signifikan dengan jumlah rata-rata 10,64 ind/ekor (sedang) sebelum transportasi dan 12,08 ind/ekor (sedang) setelah proses transportasi. Terdapat korelasi antara kadar glukosa darah dan infestasi ektoparasit dengan derajat korelasi Berdasarkan uji chi diperoleh bahwa perlakuan sistem transportasi ikan Mas Punten dapat mempengaruhi nilai kadar glukosa darah ikan yang sangat signifikan ($\text{sig} > 0,01$), namun tidak berhubungan dengan peningkatan infestasi ektoparasit yang terjadi ($.338 > 0,01$).

Kata Kunci: Ikan Mas, Kadar Glukosa Darah, Infestasi Parasit, Transportasi

Abstract

Carp is one of the most popular sources of animal protein. Aquaculture products must be distributed through a transportation system to meet the demand for carp. Improper transportation techniques will cause the condition of the fish to deteriorate, thereby increasing stress, the parasites susceptible enter the fish's body. The aim of this study was to determine the effect of transportation on blood glucose levels and ectoparasite infestation in Punten carp. The method used in this study is an experimental method with descriptive approach by comparing the values of blood glucose levels and parasite infestations in fish before the transportation process and after the transportation process. The results of the analysis of water quality before the transportation and after the transportation process still within normal limits. In the transportation treatment, Mas Punten fish can increase blood glucose levels significantly with a value before transportation of 31.54 mg/dL and after transportation of 51.41 mg/dL. The increase in ectoparasite survival was not significant with an average number of 10.64 ind/head (medium) before transportation and 12.08 ind/head (moderate) after the transportation process. There is a correlation between blood glucose levels and ectoparasite infestation with the degree of correlation. Based on the chi test, it was found that the treatment of the Punten Mas fish transportation system can affect the blood glucose level of fish very significantly ($\text{sig} > 0.01$), however it is not associated with an increase in ectoparasite infestations that occur ($.338 > 0.01$).

Keywords: Carp, Blood Glucose Levels, Parasite Infestation, Fish Transport



1. INTRODUCTION

Carp fish (*Cyprinus carpio* L.) is one of the sources of animal protein that is a prevalent commodity in Indonesia. The Punten carp fish was first cultivated in 1933 in Punten Village, Malang, East Java. The body is relatively short, but the back is wide and tall (Zubaidah *et al.*, 2021). Therefore, the body shape of the Punten goldfish seems to be bulging or short round (big belly). The ratio between total length and height is 2.3-2.4:1. The color of the scales is dark green, the eyes are slightly protruding, the body movements are slow, and they are docile (Susilo *et al.*, 2018). The distribution of aquaculture products is essential for aquaculture production to meet the demand for carp. One of the crucial factors in supporting the success of the aquaculture process is the fish transportation process.

The transportation system is a supporting facility in aquaculture activities to ensure a high level of fish survival at the destination. Carp transportation is usually carried out in live conditions. Live fish transportation technology that is in accordance with the demands of commodities and their conditions is very necessary. Generally, there are two methods of transporting live fish, namely using water as a medium or a wet system, and a medium without water or a dry system (Supriyanto and Dharmawantho, 2021).

Poor transportation techniques will cause the condition of the fish to deteriorate, thereby increasing stress and fish mortality rates while traveling or arriving at the location. Stress is a defense response in animals against a cause (stressor). Various sources of stress in the form of environmental factors (temperature, light, maintenance, capture, and transportation) as well as biotic factors such as microorganism infestations will have a negative impact on physiological changes in the animal's body (Sarimudin *et*

al., 2016; Rakhmawati *et al.*, 2018). Stress that occurs in fish can be caused by biological stressors, namely the presence of microbes (pathogenic, non-pathogenic), and procedural, namely errors in handling or transportation (Yukgehnaish *et al.*, 2020; Santos *et al.*, 2020). These two causes can cause physiological changes in fish. Under stress conditions, fish are characterized by increased glucose levels due to hormone secretion from the adrenal glands, increased respiratory rhythm, and decreased inflammatory response by hormones from the adrenal glands (Masjudi *et al.*, 2016; Santos *et al.*, 2020).

Changes in the media environment in the transportation process also cause stress to the fish so that the fish's self-defense mechanism becomes weak, and eventually disease agents such as parasites, bacteria, or viruses can easily enter the fish's body (Nasmi *et al.*, 2017; Syamsunarno *et al.*, 2019). The presence of parasites can inhibit the growth rate of the host and even directly or indirectly can kill the host. Parasites are living organisms that take advantage of other organisms as hosts. Ectoparasites are a type of parasite found outside the body of fish. Ectoparasites come from monogeneans, protozoa, and crustaceans (Timi and Poulin, 2020). Protozoan parasites are microscopic bodies consisting of a single cell membrane, and division is carried out asexually. Protozoan ectoparasites are found to infest the skin, gills, and fins of the host. Parasite infestation can occur due to an imbalance between the environment, biota, and disease-causing agents (Ulkhq *et al.*, 2018).

The problem of transportation needs special attention because one of the factors that cause many fish deaths during the trip is stress due to the environment. The aim of this study was to determine the effect of transportation on blood glucose levels and ectoparasite infestation in Punten carp. The benefit of this research is to provide

information about the relationship between blood glucose levels and ectoparasite infestation due to the transportation process in Punten carp and can be used as a reference in the management and handling of Punten carp aquaculture.

2. RESEARCH METHOD

2.1 Research Design

The research was conducted in July 2021 at the Punten Aquaculture Installation in Batu City, East Java. The method used in this research is experimental, namely by using different treatments to determine the effect of each treatment. Experimental research methods allow researchers to manipulate certain conditions to observe any effects caused by the treatment carried out during the study (Margono, 2005).

2.2 Sampling

Sampling was done randomly where all seeds in the tub have an equal chance to be taken. According to Cameron (2002), the minimum number of samples that can be represented in survey research with a population of more than 1000 is 27. Therefore, the number of samples in this study is 50 individuals.

2.3 Research Material

2.3.1 Research Tools

The tools used in this study are: Tools used to take samples (saser, plastic buckets), water quality measuring devices (DO meters, pH meters, ammonia measurement test kits), digital blood glucose levels (Blood Glucose Monitoring System Brands, GlucoDr), 1 ml syringe, tools for packing fish (plastic packing bags, oxygen cylinders, rubber, tools for examining ectoparasites (plastic trays, scissors, object, and cover glass, dropper pipette and binocular microscope).

2.3.2 Materials

The materials used in this study were 1000 fish Punten carp seeds measuring 9-10 cm, 10% EDTA (Ethylene Diamine Tetra Acid) solution, glucose test strips brand GlucoDr, aquades, syringes, label paper, plastic packing, and rubber bracelet.

2.4 Procedures

2.4.1 Fish blood sampling

Fish blood was obtained by using a 1 ml syringe filled with 10% Ethylene Diaminatetraa Acetic (EDTA) acid solution which functions as an anticoagulant. Blood was taken from the caudal vein, which is a vein located right in the heart of the fish. Suck blood slowly then the syringe is removed (Hidayaturrahmah, 2015). After that, the blood is put into a microtube that has been given anti-coagulant and label paper.

2.4.2 Fish blood glucose measurement

The fish's blood glucose levels were measured before and after transportation. Fifty goldfish in each replication were taken as blood samples from the heart of the fish. Then the blood sample that has been taken is tested with a glucose test kit and inserted at the end of the final test strip into a digital blood glucose test (Blood Glucose Monitoring System Brand GlucoDr). The results of blood glucose levels will be displayed on the device screen in the form of numbers in mg/dL units.

2.4.3 Protozoal ectoparasite examination

The examination of ectoparasites was carried out natively by scrapping the body surface, fins, and gills. Scraping is carried out from the anterior end of the head to the posterior tail fin, scraping is carried out on both sides of the fish body and also all parts of the fin (Muttaqien *et al.*, 2019). The result of scraping in the form of mucus is placed into a petri dish and dripped with distilled water. Then placed on the object glass using

a dropper, leveled, and covered with a cover glass. Examine under a microscope at 100x and 400x magnification.

The examination of ectoparasites on the gills was carried out by cutting the gills of Punten goldfish using a section tool. The gill cuttings were then separated based on the sheets and one by one placed on a glass object with a drop of distilled water until evenly distributed. Examination of ectoparasites on the gills was carried out directly under a microscope with 40x and 100x magnification (Ryani *et al.*, 2017). The results of the identification of parasites were analyzed descriptively by comparing the morphology according to the literature.

2.4.4 Water quality measurement

The water quality parameters observed included temperature, dissolved oxygen (DO), pH, and ammonia. Water measurements were carried out before and after transportation. Measurement of temperature and DO (Dissolved Oxygen) using a DO meter (Lutron Electronic Enterprise Co., Ltd.; Taiwan), measuring water pH with a pH meter (AMTAST EC910; USA Inc.), and measuring ammonia using Ammonia test kit Method: photometric 0.010 - 3.00 mg/l NH₄-N Spectroquant® (Merck, Germany).

2.5 Data analysis

The results of the examination of blood glucose and ectoparasite infestation in carp are presented in tabular form using the chi-square test to determine the relationship between the transport system with blood glucose and ectoparasite infestation in carp.

3. RESULTS AND DISCUSSION

3.1 Blood Glucose Levels

The results of measuring blood glucose levels and ectoparasite infestation in

Punten Mas (*Cyprinus carpio*) are shown in Figure 1. Based on these results, the value of blood glucose levels experienced a significant increase during the treatment before and after transportation. Meanwhile, the average value of ectoparasite infestation did not increase significantly during the treatment. Before transportation, all samples of punten goldfish (50 fish) had low blood glucose levels. However, after transportation, there were 38 animals with low glucose levels, 7 with moderate glucose levels, and 5 animals in the category of high blood glucose levels (table 1).

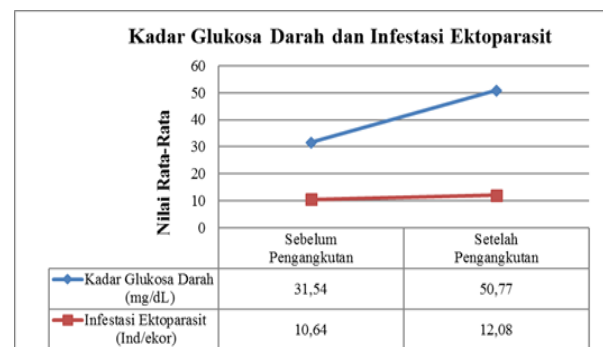


Figure 1. Blood glucose levels and ectoparasite infestation before and after transportation

Based on the results of the chi-square test, it was found that there was a relationship between transportation treatment and glucose levels, where transportation had a significant/significant effect on the value of Punten goldfish blood glucose levels with a probability value of $0.000 < 0.05$ (table 2). The average blood glucose level of Mas Punten fish before transportation is 31.54 mg/dL, and after transportation is 51.41 mg/dL.

The average value of blood glucose levels in fish before transportation is lower than when transportation is carried out. This is because at the time of transportation the fish experience stress, then they experience primary and secondary responses. Increased blood glucose is a secondary response of fish experiencing stress, after the primary response occurs, namely an increase in the number of stress hormones such as cortisol



and catecholamines from internal cells. When under stress, there is an increase in glucocorticoids which results in an increase in blood glucose levels to cope with high energy needs during times of stress (Nasichah *et al.*, 2016). This can be interpreted that fish experience higher stress than before transportation. Normal blood glucose levels for fish contain 40-90 mg/L (Fajriyani *et al.*, 2015). Meanwhile, the blood glucose level

before transportation was 31.54 mg/dL and after transportation was 51.416 mg/dL, although there was an increase in blood glucose levels, it was still in the normal range. The value of blood glucose levels can be used as an indicator of stress levels in fish. The higher the blood glucose level, the higher the stress level experienced by fish.

Table 1. Relationship between fish transportation system and blood glucose levels

		Blood Glucose Level			Number of Samples
		Low	Moderate	High	
Transportation	Before Transportation	50	0	0	50
	After Transportation	38	7	5	50
Total		88	7	5	100

Table 2. Chi square test analysis Relationship between fish transportation system and blood glucose levels

	Value	df	.sig
Chi-Square Test	13.636 ^a	2	.001 ^{*)**}
Number of Samples	100		

Keterangan: * significantly different effect (p<0,05)
 **very significant different effect (.sig<0,01)

Punten carp seeds that experience stress are indicated by the presence of high glucose levels. The seeds are in a plastic bag can cause physiological changes by shock during transportation. The increase in temperature, ammonia levels and a decrease in pH can be used as an indicator of the high organic matter in the transportation medium due to media stirring. This causes a decrease in water quality and fish to become stressed which is characterized by high glucose levels. The higher the temperature, the more agile and active the fish, requiring a higher oxygen supply (Munandar *et al.*, 2017).

High glucose levels due to receptor organs will receive information that will be sent to the hypothalamic brain, then chromaffin cells will secrete catecholamine hormones. This hormone will suppress the secretion of the hormone insulin which serves to help supply glucose into cells, causing an increase in glucose levels in the blood. This hormone will also activate enzymes involved in the catabolism of liver and muscle

glycogen stores. At the same time, the hypothalamus will release CRF (Corticotropin Releasing Factor) which will regulate the pituitary gland to secrete ACTH (Adenocortico-Tropic Hormone). ACTH functions to regulate growth mechanisms, cell metabolism, regulation, and release of cortisol. This hormone will regulate the hormone cortisol from the internal. This cortisol will bully the enzymes involved in gluconeogenesis which results in an increase in blood glucose originating from non-carbohydrate sources (Masjudi *et al.*, 2016).

3.2 Ectoparasite Infestation

Based on Figure 1 there was an increase in the infestation value of 12.08 ind/bird compared to before treatment of 10.64 ind/bird with a moderate level of ectoparasite infestation. Before transportation, there were 7 samples of punten goldfish found to be lightly infested,



and 43 fish were moderately infested. Meanwhile, after transportation, there were only 4 fish that were categorized as lightly infested and increased to 46 fish that were moderately infested (table 3). There is an increase in infestation but not significant.

Based on the results of the chi-square test, there was no relationship between the transportation process and the level of ectoparasite infestation.

Table 3. Relationship of fish transportation system with ectoparasite infestation level

	Ectoparasite Infestation Rate		Number of Samples
	Low	Moderate	
Transportation Before Transportation	7	43	50
After Transportation	4	46	50
Total	11	89	100

Table 4. Analisis Uji *Chi square* Hubungan sistem transportasi ikan dengan tingkat Infestasi ektoparasit

	Value	df	.sig
<i>Chi-Square</i> Test	.919 ^a	1	.338
Number of Samples	100		

Table 4 shows that transportation does not have a significant effect on the value of Punten carp ectoparasite infestation with a probability value of $0.338 > 0.05$, which means that transportation does not affect the level of ectoparasite infestation of Punten goldfish. So even though there was an increase in infestation, it was not caused by the fish transportation process.

Punten carp seeds that experience stress will cause the body's defense system to decrease and cause fish to be vulnerable to ectoparasite attacks which can cause high ectoparasite infestations. Changes in glucose levels in fish seeds will weaken the immune system. The disease will attack fish if there is an interaction between the host, pathogen, and the environment (Sarimudin *et al.*, 2016). This interaction causes stress in the fish, so the defense mechanism is weakened, and causes the disease to easily enter the body and cause disease. The ectoparasite infestation was still at a moderate level according to Nuriani and Suwartiningsih (2021) with an infestation of 11 ind/head being moderate.

3.3 Water Quality Measurement

Water quality measurements were carried out every day during the maintenance period. The average results of water quality measurements during the rearing period can be seen in Table 5. Water conditions as a medium for punten carp were well controlled before and during the transportation process. The value of water quality before and after transportation was found that the temperature before transportation was 27°C and after transportation was 28°C, this indicates that there was an increase in temperature after transportation. However, the temperature range is still in normal condition. The pH value was obtained at 7.6 before transportation and 7 after transportation. The DO value before and after did not change, namely 5 mg/ltr, the ammonia value increased, namely 0.05 mg/l before transportation and 0.1 mg/l after transportation. The best water quality is essential in fish cultivation to provide optimal results in aquaculture. Water quality is affected by various chemicals dissolved in water including temperature, pH, and dissolved oxygen.

The values before and after the transportation period, it is indicated that the temperature value has increased after



transportation but is still within the recommended range. The temperature has increased before transportation by 27⁰C to 28⁰C. The water temperature has increased because it adjusts to the ambient temperature during transportation. Temperature Such an increase in temperature is considered quite good, because according to (Juwahir *et al.*, 2016) that the optimal temperature range for fish life is 25-30⁰C. pH decreased to 7 this was due to an increase in CO₂, namely increased fish respiration, but was still in the recommended range. This condition is still in accordance with the pH conditions of the waters that fish need to survive. This is supported by the opinion of Kordi and Tancung (2007), which states that in aquaculture, at pH 5 fish can still be tolerated but fish growth will be hampered. However,

fish can experience optimal growth at pH 6.59. According to Zubaidah *et al.* (2021), the degree of acidity that can still be tolerated by freshwater fish is 5 mg/l. DO is still in the normal range, in fish fry transportation the oxygen content should not be less than 2 mg/l. Ammonia increased and exceeded the recommended range. The addition of ammonia levels during the transportation process is thought to come from the energy reserves of Punten carp because an increase in temperature causes fish metabolism to increase. The increase in ammonia levels in the water media of punten carp can also be caused by the results of excretion in the media during transportation trips. Fish excrete ammonia in the transportation system at 0.015 mg/L per hour (Supriyono *et al.*, 2016).

Table 5. Water Quality Value Before and After Transportation

Parameters	Before Transportation	After Transportation	Optimal Standard
Temperature (°C)	27	28	25-30*
pH	7,6	7	6,6-8,5*
DO (mg/ltr)	5	5	> 5*
NH ₃ (mg/ltr)	0,05	0,1	< 0,01*

Source : *SNI 2009 (BSN, 2009)

3.4 The relationship between the transport process with blood glucose levels and ectoparasite infestation

The chi-square or chi-square test is a non-parametric statistical test that can be used to compare two or more groups based on categorical data. However, the chi-square test can also be used for testing one group and interval/ratio scales (Ismail, 2018). In this study, a close relationship was obtained with a very significant analysis value based on the chi-square test between the transportation process and blood glucose levels. Blood glucose levels are one indicator of fish experiencing stress. This can occur due to shocks during travel, overcrowding, lack of oxygen, and fluctuating temperature changes (Andriani, 2018). Meanwhile, the results of the water media parameters show that they

are still within the optimal tolerance limit. Fish transported without stunning conditions are more likely to experience stress due to shock awareness and other activity sensors.

Based on the results of the chi-square test, shows that there is no significant relationship between the transportation process and the incidence of parasitic infestation in fish. Stressed fish can be potentially susceptible to parasitic infestations (Nasmi *et al.*, 2017; Syamsunarno *et al.*, 2019). However, parasite infestations pay attention to several things, namely, the density of fish in a medium, the presence or absence of an infestation agent, and the prevalence of the time of the infestation phase until it is infested. High fish density allows the quality of cultured water to decrease and increases direct contact between fish and other fish infested with parasites so

that parasites can spread quickly (Hasyimia *et al.*, 2016). In this study, the media and fish density per group during transportation were controlled. Thus, an increase in the number of parasitic infestations in fish was found but not significant. This can be because the main factor of fish infestation has not been sufficiently met.

4. CONCLUSIONS

In this study, it was found that the treatment of the Punten Mas fish transportation system could significantly affect the blood glucose level of fish ($\text{sig} > 0.01$), however, it was not associated with an increase in ectoparasite infestations that occurred ($.338 > 0.01$). During the transportation process, the value of the water quality of the Punten carp media was quite good and optimal.

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