BIO Web of Conferences **221**, 01009 (2026) https://doi.org/10.1051/bioconf/202622101009
7th EMBRIO International Symposium (EIS 2025)

The Effect of guava extract on water quality and survival of tilapia (*Oreochromis niloticus*) during closed transportation

Annisa Rizka Ramadhani[1], *Eddy* Supriyono[1*], *Yuni* Puji Hastuti[1], *Wildan* Nurussalam[1], *Moh* Burhanuddin Mahmud[1]

[1] Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University, 16680 Bogor, Indonesia

Abstract. Transportation activities are a major cause of tilapia mortality due to stress induced by long travel, decreased dissolved oxygen, and high levels of ammonia. The addition of guava leaf extract expected to be an alternative solution. This study aimed to analyze the effect of guava leaf extract addition during transport and post-transport rearing on survival rate, water quality, blood glucose, and specific growth rate of tilapia. This study used a completely randomized design of two treatments and one control all in triplicate. Transportation 24 hours and maintenance after transportation 21 days. The results of the study showed more optimal water quality and changes in blood glucose after transport in control 117.33 mg dL^{-1}, while 0.50% was 87.33 mg dL^{-1}, which was relatively lower than the control. A 0.50% dose resulted in an increase in survival rate (SR) from 80% in the control group to 90%. After maintenance treatment with extract resulted in an SR of 100%. Specific growth rate in treatments did not have a significant effect. Conclusion is that the addition of guava leaf extract as an environmentally friendly additive at 0.50% of the total water volume used in tilapia significantly affects SR, water quality during transportation, and blood glucose levels in fish after transportation.

1 Introduction

Tilapia (*Oreochromis niloticus*) cultivation is a freshwater fishery product businesses that has significant economic value if managed and developed properly. Stated that based on the composition of production in the fourth quarter, the fish commodity with the highest production in aquaculture is tilapia of 482.25 thousand tons, which experienced an increase in growth of 43.71%. The largest number of tilapia hatchery production in 2022 according to KKP statistical data [1]. Transportation is crucial when fish fry are distributed for further shipment to nursery or grow-out sites. Generally, transportation activities are divided into two methods, namely wet transportation and dry transportation. The transportation of tilapia fry using wet transportation uses a closed system. Usually, open transportation is used for fish for consumption and relatively close distances, while closed transportation systems are used for long-distance shipping to save space [2].

Water quality during transportation must be considered as a determinant of fish survival. Fish stress is caused by the fact that during transportation there is a reduction in dissolved oxygen content in the container, accumulation of carbon dioxide and total ammonia nitrogen, as well as the effect of temperature during the journey. In addition to fish metabolic activity, other factors that cause fish stress are density and shock during transportation. The fry quality

*Corresponding author: eddy_supriyono@apps.ipb.ac.id

is highly dependent on effective transportation methods, which are an important phase in ensuring production sustainability [3].

Efforts to reduce water quality problems during transportation can be done in several ways. For example, modification of carrier media and materials that can reduce metabolic activity during fish delivery. One of the widely used methods is the chemical MS-222/Tricaine methanesulfonate as an anesthetic [4]. The use of chemicals is feared to have a negative impact on fish and also in its use requires relatively high costs. In addition, in aquaculture products, food safety is prioritized by replacing chemicals with materials that are easily degradable in nature. One alternative natural ingredient that can be used is guava leaf extract (*P. guajava*) mixed into water medium during transportation [5].

According to [5], guava leaf extract contains secondary metabolites of flavonoids, tannins, alkaloids, steroids and saponins. Guava leaves contain flavonoids in which there is quercetin which functions to inhibit the movement of the intestinal wall in fish or antimetabolites in fish. Quercetin is directly related to suppressing the metabolic and respiration processes of fish. Efforts to suppress fish metabolism can be done to maintain water quality during the transportation process. In addition, the extract of guava leaves can be used for stress management, which can reduce the rate of fish mortality [6]. The flavonoid and saponin content is beneficial in the prevention of stress during fish transport. Saponins are also secondary metabolites used in anesthesia. However, research on the effectiveness of guava leaf extract in maintaining water quality, reducing stress, and increasing the survival rate of tilapia during 24 hours of transportation and after transportation is still limited. Therefore, this study aims to analyze the effect of adding guava leaf extract during closed-system transportation and post-transportation on survival rates, water quality, blood glucose levels, and specific growth rate.

2 Methods

2.1 Time and place

This study in November – December 2023. This study was conducted at the Aquaculture Environmental Laboratory 3 and water quality at the Aquaculture Environmental Laboratory 1, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University.

2.2 Test materials

The tilapia used is obtained from cultivators in Tegal Waru, Bogor Regency. The fish used had an average length of 6.76±0.06 cm and an average weight of 5.70±0.08 g. The guava leaves used are located around the IPB Dramaga campus with no physical damage taken from the same guava tree. When making guava extract solution, use freshly picked leaves. The extract was then prepared using the boiling method. Guava leaves were blended, then aqua destillata was added at a ratio of 1:16. The extraction process continued by boiling until only ¼ of the initial volume remained. The boiled mixture was then filtered using filter paper [7]. The filtered result was made into a guava leaf extract solution, which was then diluted to the desired concentrations of 0%, 0.25%, and 0.50%.

2.3 Research design

This method is Completely randomized design (CRD) with two treatments and one control. Each of these treatments has three repeats. Each plastic bag contains 20 fish L^{-1}. Each bag contains 2 L^{-1}. The dosage refers to the previous lethal concentration (LC_{50}) in Table 1.

Table 1. Dosage of guava leaf extract used during transportation.

Treatment (Dosage)	Information
K (Control)	0% of the total volume of transportation water used
JB1 (Guava 0.25%)	0.25% of the total volume of transportation water used
JB2 (Guava 0.50%)	0.50% of the total volume of transportation water used

2.4 Test parameters

2.4.1 Survival rate

Fish survival is observed during transport treatment and after transport. In addition, during maintenance keep an eye on the survival of the fish. The formula to find out SR is:

$$SR\ (\%) = Nt/No \times 100 \quad (1)$$

where: *SR* = Survival Rate (%), *Nt* = Number of fish at the end of the cultivation period (individuals), *No* = Number of fish at the beginning of the cultivation period (individuals).

2.4.2 Blood glucose

Blood glucose levels of tilapia fry were measured before, after transportation, and one week after transportation with extract concentrations of 0%, 0.25%, and 0.50% were performed. Three fish in each treatment were taken as samples for blood collection from the caudal vein using a 1 ml syringe [8]. Three samples because to minimize stress and over mortality after transport since the fish are not anesthetized due to their small size, blood sampling is performed quickly and carefully to minimize stress and to avoid the effects of anesthetics when observed during maintenance. The blood is then tested with a glucose level test kit (*Gluco Easy Touch code: 2692*).

2.4.3 Specific growth rate

The specific growth rate represents the percentage increase in fish body weight per day. It is determined using the following formula:

$$SGR(\%/day) = [(ln(Wt) - ln(Wo)/t] \times 100 \quad (2)$$

where: *SGR*=Specific Growth Rate (%/day), *Wt*=Average weight of fish at the end of the study (g), *Wo*=Average weight of fish at the beginning of the study (g), *t*=Time period (days), *ln*=Natural logarithm.

2.4.4 Water quality

Water quality measurements are carried out during transportation and maintenance. The parameters tested at the time of transportation are DO, pH, temperature, nitrite, TAN, and during maintenance DO, pH, temperature, nitrite, TAN, alkalinity, and hardnees.

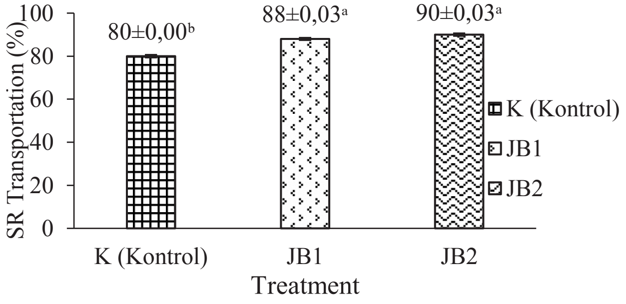
Table 2. Water quality parameters observed.

Parameters	Unit	Method	Time	
			Transportation	**Maintenance**
DO	mg L^{-1}	SNI 06-6989.14:2004	6 hours once	Once a day
pH	-	SNI 6989.11:2019	6 hours once	Once a day
Temprature	^{o}C	SNI 6989.23-2005	6 hours once	Once a day
TAN	mg L^{-1}	APHA, ed. 21, 2005,4500-NH_3-F	6 hours once	once a week
Nitrite	mg L^{-1}	APHA, ed. 21, 2005,4500-NO_2-B	6 hours once	once a week
CO_2	mg L^{-1}	IK Titimetri	6 hours once	once a week
Alkalinity	mg L^{-1} $CaCO_3$	IK Titimetri	—	once a week
Hardnees	mg L^{-1} $CaCO_3$	SNI 06-6989.12-2004	—	once a week

3 Results

3.1 Survival rate transportation

The following is the percentage of tilapia survival rate transportation which can be seen in Figure 1.



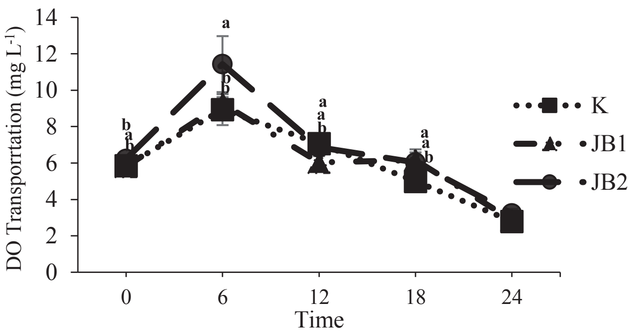
*Different superscript letters indicate significant differences ($p<0.05$).

Fig. 1. The survival in tilapia transport water with different guava leaf extract concentrations for 24 h (K: control, JB1: 0.25%, JB2: 0.50%).

Based on the ANOVA test, SR transportation in the K treatment was significantly different ($P<0.05$) from the JB1 and JB 2 treatments, but JB1 and JB2 were not significantly different ($P>0.05$).

3.2 Dissolved oxygen (DO) transportation

Figure 2 shows the dissolved oxygen (DO) levels in the tilapia transportation medium.



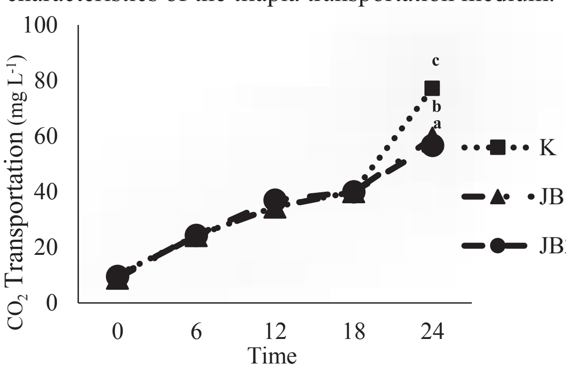
*Different superscript letters indicate significant differences (P<0.05).

Fig. 2. Dissolved oxygen in tilapia transport water with different guava leaf extract concentrations for 24 h (K: control, JB1: 0.25%, JB2: 0.50%) .

Dissolved oxygen (DO) levels in all treatments increased at 6 hours and then decreased from 12 to 24 hours. Based on the ANOVA test, the transport DO values at hours 0, 6, 12, and 18 were significantly different (P<0.05), but at the 24 hour there was no significant difference (P>0.05).

3.3 Carbon dioxide (CO_2) transportation

Figure 3 shows the characteristics of the tilapia transportation medium.



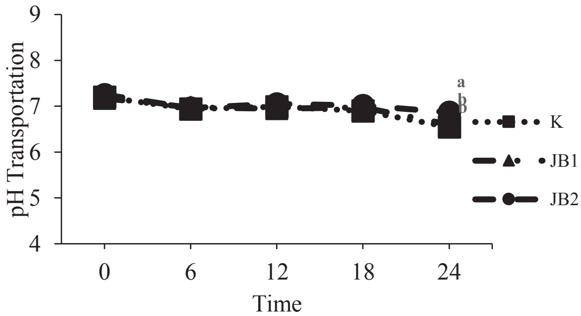
*Different superscript letters indicate significant differences (P<0.05).

Fig. 3. Carbon dioxide in tilapia transport water with different guava leaf extract concentrations for 24 h (K: control, JB1: 0.25%, JB2: 0.50%).

The CO_2 value in each treatment continues to increase as the transportation time increases. Based on the ANOVA test, the transportation CO_2 value at the 0, 6, 12, 18th hour was not significantly different (P>0.05) and the 24th hour was significantly different (P<0.05) for each treatment.

3.4 pH transportation

The following is the pH of tilapia transportation medium transportation which can be seen in Figure 4.



*Different superscript letters indicate significant differences (P<0.05).

Fig. 4. pH in tilapia transport water with different guava leaf extract concentrations for 24 h (K: control, JB1: 0.25%, JB2: 0.50%.

Based on the ANOVA test, the pH of transportation at the 0, 6, 12, 18th hour was not significantly different (P>0.05) and the 24th hour was significantly different (P<0.05) for each treatment.

3.5 Temperature transportation

The following is the temperature of tilapia transportation media transportation which can be seen in Figure 5.

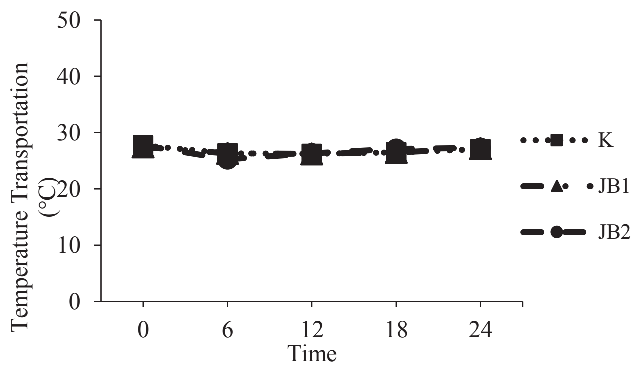
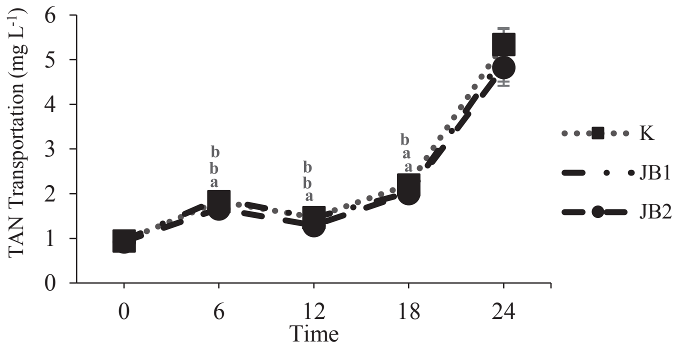


Fig. 5. Temperature in tilapia transport water with different guava leaf extract concentrations for 24 h (K: control, JB1: 0.25%, JB2: 0.50%).

Based on the ANOVA test, the transportation temperature at hours 0, 6, 12, 18, and 24 were not significantly different (P>0.05) for each treatment. The temperature range transportation is 25.3-27.7 ºC.

3.6 TAN Transportation

Figure 6 shows the total ammonia nitrogen (TAN) concentration in the tilapia transportation medium.



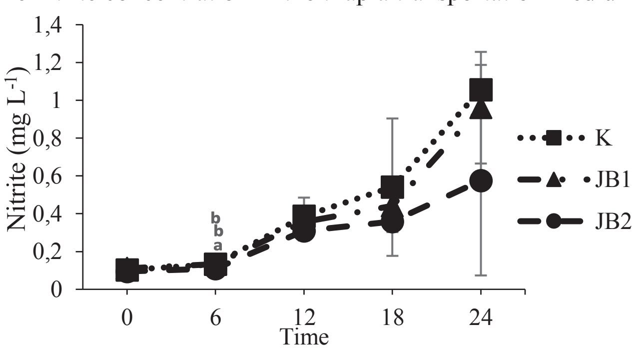
*Different superscript letters indicate significant differences (P<0.05).

Fig. 6. TAN in tilapia transport water with different guava leaf extract concentrations for 24 h (K: control, JB1: 0.25%, JB2: 0.50%).

Based on the ANOVA test, the of transportation TAN at the 6, 12 and 18 hours was significantly different (P<0.05) and at 0 and 24 hours there was no significant difference (P>0.05).

3.7 Nitrite transportation

Figure 7 shows the nitrite concentration in the tilapia transportation medium.



*Different superscript letters indicate significant differences (P<0.05).

Fig. 7. Nitrite in tilapia transport water with different guava leaf extract concentrations for 24 h (K: control, JB1: 0.25%, JB2: 0.50%).

Based on the ANOVA test, the of transport nitrite at hours 0, 12, 18, and 24 was not significantly different (P>0.05), but at the 6th hour it was significantly different (P<0.05).

3.8 Survival rate after maintenance

The following is the percentage value of tilapia survival rate after maintenance which can be seen in Figure 8.

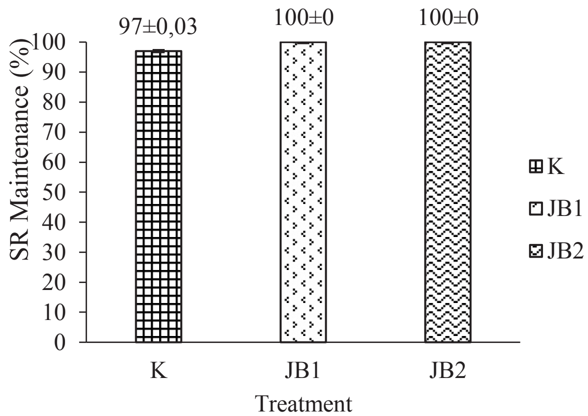


Fig. 8. Survival rate of tilapia fry after 21 days of rearing.

The maintenance SR of K, JB1, JB2 treatment was not significantly different (P>0.05). The JB1 and JB2 treatments did not result in death, while controls resulted in 3% of the initial stocking after transport.

3.9 Water quality after maintenance

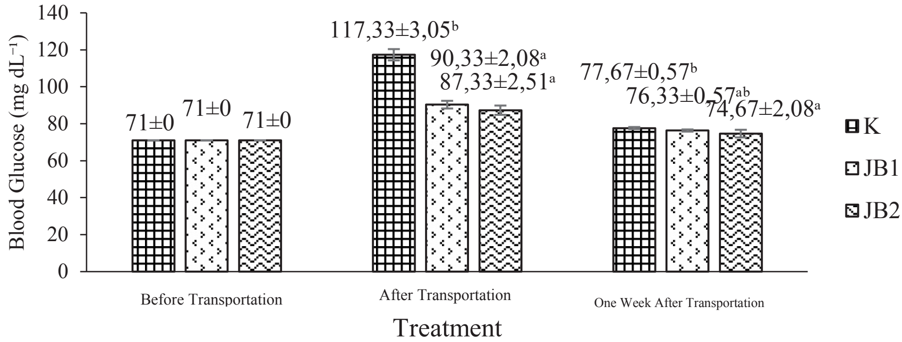
The values of water quality parameters in situ (DO, pH, temperature) and ex situ (Nitrite, TAN, CO_2, alkalinity, and salinity) during 21 days of maintenance after transportation are shown in Table 3.

Table 3. Value of maintenance water quality parameters after transportation.

Parameters	Range values			Optimal value	Source
	K	JB1	JB2		
DO	6.63 — 9.17	6.80 — 9.17	6.83 — 9.23	>5 mg L^{-1}	[9]
CO_2	5.33 — 7.99	4.00 — 7.99	4.00 — 7.99	<15 mg L^{-1}	[9]
pH	6.21 — 8.48	6.44 — 8.47	6.12 — 8.51	6.5 — 8.5	[9]
Temperature	25.90 — 27.03	26.03 — 27.23	25.97 — 27.07	23 — 30 ^{oC}	[9]
TAN	0.13 — 0.17	0.12 — 0.14	0.11 — 0.14	<0.02 mg L^{-1}	[9]
Nitrite	0.10 — 0.12	0.09 — 0.11	0.09 — 0.11	<1 mg L^{-1}	[9]
Alkalinity	41.33 — 46.67	42.67 — 48.00	41.33 — 49.33	20 — 300 mg L^{-1}	[9]
Hardness	41-37 — 44.04	44.04 — 48.05	44.04 — 52.05	20 — 300 mg L^{-1}	[9]

3.10 Blood glucose

The following is the of blood glucose measured before transportation, after transportation, and after a week of maintenance in tilapia is shown in Figure 9.



*Different superscript letters indicate significant differences (P<0.05).

Fig. 9. Blood glucose of tilapia fry in three treatments before transportation, after transportation, and after a week of maintenance.

Based on the results of the ANOVA test, the blood glucose after transport of treatment K were significantly different (P<0.05) with JB1 and JB2, but JB1 and JB2 were not significantly different (P>0.05), and after a week of maintenance treatment K was significantly different (P<0.05) with JB2 but JB1 was not significantly different (P>0.05) from K and JB2. JB2 reduced glucose levels significantly vs. control, indicating lower stress.

3.11 Specific growth rate

The following is the percentage of the specific growth rate of tilapia during maintenance is shown in Figure 10.

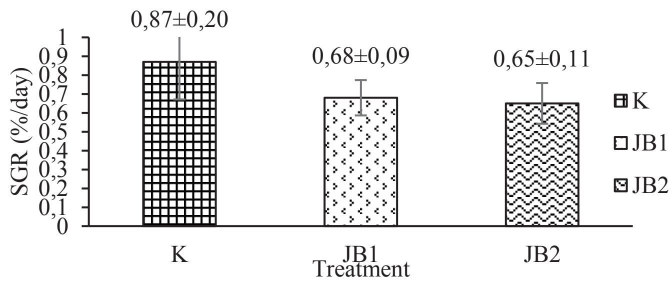


Fig. 10. Value of the specific growth rate of tilapia after 21 days of rearing.

Based on the results of the ANOVA SGR test, K, JB1 and JB2 treatment were not significantly different (P>0.05).

4 Discussion

Indicators of stress levels in fish can be correlated with increased blood glucose levels that occur from measurements at the beginning of transport and after simulations are conducted. The optimal level for fish blood glucose ranges from 40 ─ 90 mg dL^{-1}. Indicate the level of stress of fish, the results of blood sugar content measurement can be carried out. Increased blood glucose levels are a secondary effect of stress, namely through the release of corticosteroids and catecholamines. Stressful conditions can cause an increase in glucocorticoids which results in an increase in blood glucose levels used to overcome high energy needs [10]. Based on the results of blood glucose measurements, the administration of guava leaf extract resulted in blood glucose levels during transport compared to controls without being given extract. The addition of guava leaf extract to the JB1 and JB2 treatment is considered to reduce the level of glucose use in the blood for homeostasis activities against environmental stress. Supported by the opinion of [5], guava leaves contain secondary metabolites that are able to suppress stress levels and as an antihyperglycemic in fish.

The Stocking density in the container is a crucial factor that influences fish stress levels and subsequently affects survival during transportation. The application of unsuitable transportation methods can negatively affect post-transport conditions, thereby increasing mortality rates at arrival and during the post-arrival period, it was carried out in a 24-hour transportation simulation [11].

Water quality during transportation can have an effect on the survival rate of fry. Dissolved oxygen concentrations in the water during this transportation will decrease along with the length of transportation [12]. DO and CO_2 play an important role in the survival of transported fish. A decrease in DO levels will increase CO_2 during the transportation process. Fish, as poikilothermic organisms, fish are highly affected by external stressors such as temperature, salinity, and water quality, which can cause stress levels [13]. The pH concentration indicates a decrease or is categorized as relatively acidic with a range of 6.5–7.3. This can be caused by the addition of guava leaf extract which contains 9–12% tannin compounds. The temperature of all treatments showed a range of 25.3–27.7°C. Temperature is important for fish health, especially for fish physiological processes. A similar study conducted by [14], Recent studies have shown that Nile tilapia can tolerate elevated ammonia concentrations during short-term exposure; however, prolonged exposure, even at relatively low ammonia levels, can induce significant metabolic disturbances. Therefore, maintaining TAN concentrations at low levels is crucial to support fish welfare and growth during transportation. Nitrite and nitrate compounds are the result of the decomposition of ammonia compounds, both photoautotrophs by algae and autotrophs and heterotrophs by bacteria. The results obtained from JB1 and JB2 treatment are still within optimal limits when compared to optimal levels according to [15], the optimal level for fish is <1 mg L^{-1} and nitrites will be toxic to fish if it reaches a level of 5 mg L^{-1}.

Furthermore, maintenance activities were carried out after transportation for 21 days). These results show that after transportation, tilapia can adapt to the environmental conditions where they are reared. Although there was a 3% decrease in controls, JB1 and JB2 had no significant effect or further effect of administration of guava leaf extract during post-transport maintenance on survival rates. Based on the results of measuring water quality parameters during the 21 days of overall maintenance, it can be said that the water conditions are suitable and optimal for the maintenance of tilapia fry. The SGR value from the 21 day maintenance results showed the highest growth produced in K. because the stocking density used in the study was in accordance with the TKH after transportation, which was as follows: (K: 80%, JB1: 88%, JB2: 90%). Stating that increasing stocking density can result in a decrease in fish growth. Competition will move space, affect the competition and physiology of the fish. Competition in obtaining feed evenly will be smaller. This competition of motion space makes fish unable to optimally utilize feed so that fish growth is disrupted and slow.

5 Conclusion

The addition of 0.50% guava leaf extract to the total volume of transport water used for tilapia not only serves as a cost-effective and environmentally friendly additive but also has a significant effect on survival rates, water quality during transport, and blood glucose levels in fish after transport. A 0.50% dose resulted in an increase in survival rate during transport from 80% in the control group to 90%, and after 21 days of maintenance, the 0.25% and

0.50% doses yielded a survival rate of 100% compared to the control group. Specific growth rate (SGR) in all treatments did not show a significant effect.

References

1 KKP, Rilis data kelautan dan perikanan triwulan IV tahun 2022. Jakarta: Pusat Data, Statistik dan Informasi KKP (2022).

2 M. K. Maraja, N. Salindeho, J. Pongoh. Penanganan ikan nila (*Oreochromis niloticus*) hidup dengan dengan menggunakan es sebagai pengawet. MTHP. **5**, 3 (2017). https://doi.org/10.35800/mthp.5.3.2017.16849

3 E. Supriyono, R. Anandasari, K. Adiyana, L. Thesiana, R. Rasidi, S. Hastuti, The effectiveness of eugenol in mitigating water quality, biochemical, and histopathological alterations during transportation of giant freshwater prawn juveniles. Iraqi j. vet. sci. **39**, 2 (2025). https://doi.org/10.33899/ijvs.2025.157578.4131.

4 L.A. Espinoza-Ramos, Y. Puma-Vilca, J.1. Huanacuni, R. Pepe-Victoriano, R. Evaluation of Tricaine (MS-222) and Eugenol for Sedation of Peruvian Grunt Anisotremus scapularis. *Animals.* **15**, 9 (2025). https://doi.org/10.3390/ani15091322.

5 I. Batubara, I.H. Suparto, N.S. Wulandari. The best extraction technique for kaempferol and quercetin isolation from guava leaves (*Psidium guajava*). Earth Environ. Sci. **58**, 1 (2017). https://doi.org/10.1088/1755-1315/58/1/012060

6 I.W. Sudira, I.M Merdana, S.N. Qurani, Preliminary phytochemical analysis of guava leaves (*Psidium guajava L.*) as antidiarrheal in calves. ATBES. **3**, 2 (2019). https://doi.org/10.24843/atbes.v03.i02.p01.

7 T Birdi, P Daswani, S Brijesh, P Tetali, A Natu, N Antia. Newer insights into the mechanism of action of *Psidium guajava* L. leaves in infectious diarrhea. BMC Complement. Med. Ther**.** **10**, 33 (2010). https://doi.org/10.1186/1472-6882-10-33.

8 T. Yuniastutik, Observasi waktu maksimal penyimpanan darah terhadap nilai hematokrit ikan lele (*Clarias* sp.). Temapela. **4**, 2 (2021).

9 SNI, Standardisasi Nasional Indonesia, Produksi Ikan nila (*Oreochromis niloticus Bleeker*) kelas pembesaran di kolam air tenang. Jakarta: SNI (2009).

10 T.N. Widianto, I. Malhani, N. Priyanto, Simulasi transportasi ikan nila hidup menggunakan sistem basah terbuka pada suhu rendah. JPBKP. **17**, 1 (2022). https://doi.org/10.15578/jpbkp.v17i1.791.

11 Y. Yang, E. Narayan, S.R. Planellas, C.Z. Phillips, L. Zheng, B. Xu, L. Wang, Y. Liu, Y. Sun, G. Sagada, H.Y. Shih. Effects of stocking density during simulated transport on physiology and behavior of largemouth bass (*Micropterus salmoides*). J. World Aquacult. Soc. **55**, 2 (2024). https://doi.org/10.1111/jwas.13054

12 D. Fang, J. Mei, J. Xie, W. Qiu. The effects of transport stress (temperature and vibration) on blood biochemical parameters, oxidative stress, and gill histomorphology of pearl gentian groupers. Fishes. **8**, 4 (2023). https://doi.org/10.3390/fishes8040218

13 H.T. Ferdinand, E. Supriyono, T. Budiardi, M. Setiawati, Ridwan, The secondary stres responses of transported glass eel (*Anguilla bicolor bicolor*) with various packing densities. AACL Bioflux. **15**, 2 (2022).

14 G. Stephen, R.E. Edziyie, K. A. Obirikorang, D. Adjei-Boateng, P. Vilhelm Skov. Nile tilapia (*Oreochromis niloticus*) show high tolerance to acute ammonia exposure

but lose metabolic scope during prolonged exposure at low concentration. Aquat. Toxicol. **27**, 1 (2022). https://doi.org/10.1016/j.aqrep.2022.101325

15 D. Deswati, E. Yani, S. Safni, O. N. Tetra, H. Pardi, Development methods in aquaponics systems using biofloc to improve water quality (ammonia, nitrite, nitrate) and growth of tilapia and samhong mustard. Int. J. Environ. Anal. Chem. **1**, 11 (2020) https://doi.org/10.1080/03067319.2020.1839437