



Mustika Carp (*Cyprinus carpio*): Growth Performance Profile of a Superior Strain Resistant to KoiHerpes Virus

Yogi Himawan¹, Didik Ariyanto², Suharyanto³, Khairul Syahputra⁴, Flandrianto Sih Palimirmo⁵,
Listio Dharmawantho⁶, Supriyanto⁷

^{1,2,3,4,5,6}Pusat Riset Perikanan, Badan Riset dan Inovasi Nasional
Jl. Raya Bogor KM.47, Kab. Bogor, Jawa Barat, Indonesia

⁷Pusat Riset Teknologi Tepat Guna, Badan Riset dan Inovasi Nasional
Jl. Aipda KS Tubun No. 5 Subang, Jawa Barat, Indonesia

E-mail : ¹yogi011@brin.go.id, ²didi031@brin.go.id, ³suha041@brin.go.id

Abstract - The outbreak of Koi herpesvirus (KHV) in carp farming has significantly reduced national carp production. Several effort have been taken to reduce this disease, one of which is the development of Mustika carp (Rajadanu carp super KHV-resistant) through selective breeding. The aim of this study is to analyze the growth performance of Mustika carp through field trials during the grow-out phase. The test subjects were Mustika carp seedlings selected for positive MHC (Major Histocompatibility Complex), while the control group consisted of carp seedlings from local farmers, each with three replications. The trial location was at the Research institute for Fish Breeding in Sukamandi, Subang Regency. Additionally, grow-out research was conducted in running water ponds in Tanjungsang, Subang; as well as floating net cages in Jatiluhur Reservoir, Purwakarta; Cirata Reservoir, Cianjur; and Darma Reservoir, Kuningan. The research was carried out with three replications over 90 days. The results showed that Mustika carp had a relatively better growth performance, with a specific growth rate of 16.83% ($2.29 \pm 0.04\%$) and a survival rate of 3.80% ($49.13 \pm 6.88\%$). In the grow-out locations, Mustika carp had a significantly better productivity of 13.42 kg/m² compared to the control group, which had 8.16 kg/m²

Keywords: mustika carp, koiherpes virus, growth performance, productivity

I. INTRODUCTION

Common carp is one of the popular freshwater fish commodities cultured especially in the regions of Java, Sumatra, Nusa Tenggara, and Kalimantan. Specifically in West Java, common carp is one of the popular freshwater commodities cultured and a mainstay in aquaculture activities, whether in earth ponds, running water ponds, and floating net cages. However, according to Ariyanto et al (2018), the decline in growth performance of several cultured fish, including common carp, has occurred over the past decade. This is indicated by the low growth of common carp and its high susceptibility to biotic and abiotic pressures such as diseases and environmental changes (Ariyanto et al, 2022). This condition is partly due to the outbreak of Koiherpes Virus (KHV) disease around 2002, which caused a drastic decline in common carp farming efforts. KHV disease is a highly contagious disease-causing agent in common carp (Monaghan et al, 2022). This also resulted in most common carp farmers in several production centers switching to other commodities.

An approach that can be taken to address KHV disease and facilitate the correlation between genetic variation and disease resistance traits is the development of disease-resistant strains through selection methods (Gao et al, 2023). One strain of common carp that has resistance to KHV disease is the Mustika carp (Rajadanu Super Resistant to KHV infection). The Mustika carp is the result of molecular marker-based selection for the KHV disease resistance trait (Ariyanto et al, 2020). The Mustika carp was developed and formed at the Research Institute for Fish Breeding (RIFB) Sukamandi, Subang Regency, West Java. Performance testing for growth and resistance to KHV was also conducted at several culture locations, including earth ponds, running water ponds, and floating net cages.

II. The Mustika carp is also a superior strain obtained through molecular marker-based selection (Marker Assisted Selection). The molecular genetic marker used in the selection activities of individual carp for the KHV resistance trait is the MHC-II gene (Major Histocompatibility Complex), specifically the Cyca-DAB-1*05 allele (Syahputra et al, 2016). Based on this, the specific aim of the research is to analyze the performance of the Mustika carp during the grow-out phase in the RIFB test ponds and at several different culture locations.

II. MATERIAL AND METHODS

Performance test on earth pond

Growth performance testing in the pond utilized concrete tanks measuring 2.5 m x 2 m x 1 m with a mud bottom. These concrete tanks were located at the RIFB in Sukamandi, Subang Regency, which is a lowland area at an altitude of 16 meters above sea level. Water quality parameters in the concrete tanks included temperature, pH, and oxygen content, which respectively ranged from 27.2-29.0 °C; 7.8-8.0; and 0.8-3.6 mg/L. The Mustika common carp seed used were obtained from spawning male Rajadanu F2 broodstock selected based on weight, and female Rajadanu F1 broodstock selected for disease resistance using MHC molecular markers through the Polymerase Chain Reaction (PCR) method. Spawning was conducted using artificial spawning methods with the aid of HCG (Human Chorionic Gonadotropin) hormone, which was injected intramuscularly into the dorsal area. The Mustika carp fry were reared until they reached a weight of 10-12 g per fish. The control fish population was obtained from local farmers in the Sukamandi area, with an average weight of 10 g per individual.

The test subjects at the experimental site consisted of two populations of common carp: (A) Mustika carp and (B) carp from local farmers commonly used. The testing used six units of 50 m² ponds with a stocking density of 10 fish/m². The trial was conducted over three months with feeding consisting of commercial pellets containing 30%-32% of protein, given twice a day. Data analysis of the research results was conducted using Chi-Square analysis to determine differences between treatments.

Multilocation test

Multilocation testing was conducted following the procedures outlined in Ariyanto et al. (2019a). Mustika carp and control fry used at the time of stocking were two months old after hatching, with an average body length of 12.05 ± 1.34 cm per fish and a body weight of 14.28 ± 4.44 g per fish. The testing locations included a running water pond (KAD) in Tanjungsang, Subang Regency, and floating net cages (KJA) in three reservoirs: Jatiluhur Reservoir in Purwakarta Regency, Cirata Reservoir in Cianjur Regency, and Dharma Reservoir in Kuningan Regency. These testing locations are situated at various elevations. The running water pond in Tanjungsang, Subang Regency, is located at an altitude of approximately 700 meters above sea level, Jatiluhur Reservoir in Purwakarta Regency at 110 meters above sea level, Cirata Reservoir in Cianjur Regency at 220 meters above sea level, and Dharma Reservoir in Kuningan Regency at 715 meters above sea level. The running water pond in Tanjungsang measures 3 m x 15 m x 2.5 m and was stocked with 7,500 fish per pond. The size of the floating net cages used at the three locations was 7 m x 7 m x 3 m. The stocking density of carp in Cirata and Dharma Reservoirs was 5,000 fish per plot, while in Jatiluhur Reservoir it was 7,500 fish per plot. During the 90 day testing period, the fry were fed by commercial sinking pellets with a crude protein content of 28%-30% by ad libitum method.

III. RESULT AND DISCUSSION

The phenotypic performance observed in each carp population maintained for three months in the test ponds showed that the Mustika carp population had better growth performance compared to the carp from local fish farmers. Analysis of the specific growth rate character showed a significant difference ($p < 0.05$) between the Mustika population and the control (Figure 1).

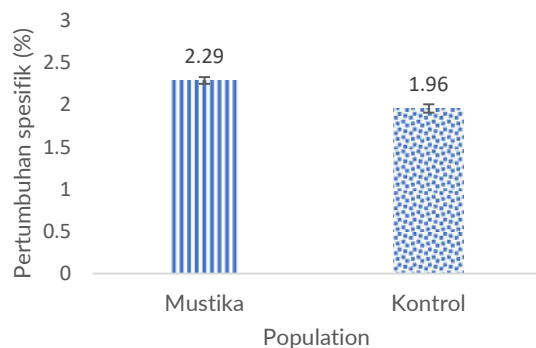


Figure 1. Graph of Average Specific Growth Rate of Mustika Carp in Concrete Tanks.

Observations on the specific growth rate characteristic showed that Mustika carp had an average value of $2.29 \pm 0.04\%$, which was higher than the control population at $1.96 \pm 0.05\%$. This is likely due to the contribution of the selected genotype from selective breeding, resulting in relatively stable performance and a positive impact on weight growth during the rearing period. Selective breeding has been widely used in fish to achieve more advantageous phenotypes (Brito et al., 2017). Under strong positive selection pressure, selective breeding can gradually increase the frequency of favorable alleles in the genome over time. According to Song et al. (2023), genomic regions with unique genetic features observed are defined as selection signatures. In selective breeding, improvement of growth-related traits is economically important and contributes to the long-term sustainability of the aquaculture industry. Additionally, the adaptability of the Mustika carp population to the rearing environment was relatively higher compared to the control, allowing energy for growth to remain undisturbed by the need to adapt to the rearing environment.

Survival rates in the test of the two populations in the RIFB Sukamandi pond showed that the Mustika population had a survival rate of $49.13 \pm 6.88\%$, which was relatively better compared to the control population, which reached $47.33 \pm 0.95\%$ (Figure 2).

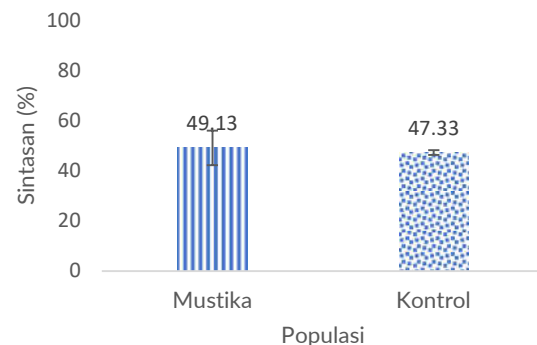
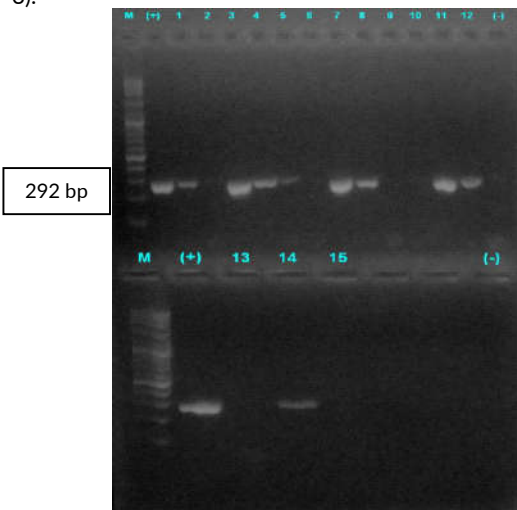


Figure 2. Graph of Average Survival Rate of Mustika Carp in Concrete Tanks.

The survival rates between populations still show relatively high average values. One reason for this could be that the fish density in this study was relatively low, so competition for feed was still within a normal range. Nhan (2020) and Nhan et al. (2022) reported that stocking density affects variations in survival, growth, and economic efficiency in seabass (*Lates calcarifer*) farming in earthen ponds. Additionally, the ability of Mustika common carp to survive by adapting to the conditions of the farming pond, as well as its biotic and abiotic factors, is presumed to be better compared to the control, resulting in higher survival rates. This is also thought to be related to the stress management ability of Mustika carp in response to environmental conditions. According to Lee et al. (2018) and Zhang et al. (2020), a fish's ability to respond to environmental stress can enhance its survival rate. Moreover, the similar survival rates might be due to the fact that Mustika carp is primarily designed to be more resistant to KHV virus attacks (Himawan et al., 2017). Under normal conditions without KHV, its survival performance is not significantly different from regular common carp, although it still tends to be higher.

Laboratory test results on samples of fish that experienced mortality using the PCR method found that 80% were positive due to KHV, while for Mustika carp, only 60% were positive, with the remainder attributed to environmental factors (Figure 3).



Note: Numbers 1-5 represent the control population, while 11-15 represent Mustika carp.

Figure 3. Results of Electrophoresis Of KHV Challenge Test Samples on A Laboratory Scale.

These results show that the Mustika population is better able to defend itself from KHV attacks than control fish, so it is hoped that genotypically this condition can continue to be maintained. Test results using the PCR method showed that as many as 80% of the control fish samples that died were

positive for KHV, so it is suspected that resistance to KHV was lower than the Mustika population, which reached 60% positive for KHV. The resistance to KHV of the Mustika population is thought to be due to the presence of MHC genes and the influence of selection activities in previous generations so that the genotype for resistance to KHV is higher than without selection.

The presence of MHC genes in Mustika carp will be very helpful in maintaining survival when KHV begins to actively attack. This is thought to be related to the specific resistance of Mustika carp to KHV attacks. The specific defense system has the ability to recognize objects that are considered foreign to itself. The specific immune system consists of humoral, cellular, and lymphoid organs. MHC is a specific adaptive immune system and is located on chromosome 6 and plays a role in recognition and signaling between immune system cells. MHC genes process and present pieces of foreign microbes that have been destroyed on the surface of infected cells so that they are easily recognized by the immune system (Rakus et al., 2008). With this introduction, the fish's body becomes better prepared to face pathogen attacks so that physiologically it is more resistant to disease. Mustika goldfish have resistance to KHV disease with survival in the challenge test amounting to 98.89% (Syahputra et al., 2016).

Growth performance in multilocation

The results of further analysis of the performance of final weight, survival and productivity of Mustika carp showed that it was significantly better than control carp in Dharma Reservoir (Table 1). However, the performance of Mustika and control carp was not significantly different ($p > 0.05$) at the other three test locations.

Table 1. Mustika Carp Culture Parameters for 90 Days in Four Different Locations

Strain	Aquaculture parameters	Location			
		Jatiluhur	Cirata	Tanjungsiang	Darma
Mustika	Final weight	312.56 ± 71.58 ^a	279.90 ± 61.91 ^a	231.84 ± 31.75 ^a	180.96 ± 36.28 ^a
	Survival rate	31.67 ± 9.50 ^a	75.00 ± 7.00 ^a	70.00 ± 7.00 ^a	75.00 ± 13.23 ^a
	Productivity	5.51 ± 7.96 ^a	21.12 ± 5.54 ^a	24.18 ± 2.08 ^a	13.42 ± 2.42 ^a
Control	Final weight	266.30 ± 62.81 ^a	224.88 ± 40.06 ^a	224.67 ± 58.34 ^a	114.79 ± 8.97 ^b
	Survival rate	35.33 ± 6.43 ^a	74.67 ± 5.03 ^a	60.00 ± 15.00 ^a	70.67 ± 9.50 ^a
	Productivity	14.08 ± 4.22 ^a	16.83 ± 3.37 ^a	19.95 ± 6.35 ^a	8.16 ± 1.71 ^b

Source: Ariyanto et al, 2019b

The faster growth rate of Mustika carp compared to control carp in the Dharma Reservoir is thought to be caused by two things. The first thing is that Mustika carp are resulting from selection, while the control carp used as a comparison are fish that come from community hatchery units, which did not go through a selection program. Although the Mustika is a carp that resulted from selection for markers related to the character of resistance to KHV disease, its implementation is also accompanied by selection for growth characteristics (Ariyanto et al, 2014). The second suspicion is that there is an increase in the tolerance of Mustika carp to low quality aquatic environments. According to Himawan et al (2022), preventing the death of carp cultured in rearing centers can be done by improving the performance of carp, one of which is through the use of carp seeds that can withstand environmental stress. The increase in the tolerance of Mustika carp is thought to be the impact

of increasing resistance to disease infections in the carp population. Although the survival value of Mustika carp was not significantly different from that of control carp at all test

locations, the final weight and productivity of Mustika carp in Dharma Reservoir were significantly better than control carp.

Distribution

In general, Mustika carp have distribute to several regions in Indonesia, including the islands of Sumatra, Java, Nusa Tenggara and Sulawesi (Figure 4). The number of prospective broodstock distributed has reached 7000 fish, and it is hoped that this will be able to increase carp production again on a national scale.

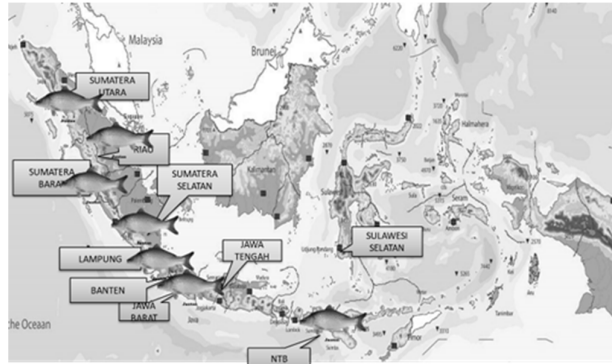


Figure 4. Broodstock distribution of Mustika carp in Indonesia

IV. CONCLUSION

The Mustika carp population had 16.83% better growth performance in specific growth rate characteristics ($2.29 \pm 0.04\%$); and 3.80% in survival characteristics ($49.13 \pm 6.88\%$) compared to controls. In several rearing locations, Mustika carp have a productivity of 13.42 kg/m², better than the control of 8.16 kg/m².

REFERENCES

- Ariyanto, D., Hayuningtyas, E.P., & Syahputra, K. (2014). Response to individual selection for growth characteristics in the F₀ population of common carp of Rajadanu strain. *J Res Aquaculture*. 9(3), 353-361.
- Ariyanto, D., Carman, O., Soelistyowati, D.T., Zairin, M., & Syukur, M. (2018). Phenotype and genotype characteristics of five strains of common carp from West Java and Banten Province. Indonesia. *J Res Aquaculture*. 13(2), 93-103 [In Indonesia].
- Ariyanto, D., Himawan, Y., Syahputra, K., Palimirmo, F.S., & Suharyanto. (2019a). Improvement of growth characteristics in "Mustika" common carp through selection. *J Res Aquaculture*. 14(2), 71-76.
- Ariyanto, D., Himawan, Y., Syahputra, K., Palimirmo, F.S., Suharyanto. (2019b). Growth performance and productivity of Mustika strain common carp in multi-location trials. *J Res Aquaculture*. 14(3):139.
- Ariyanto, D., Syahputra, K., Himawan, Y., Sih Palimirmo, F., Haryadi, J. (2020). Adaptability and production stability of common carp strain "Mustika" cultured in different farming systems and sites. *J Res Aquaculture*. 15(4):221-227.
- Ariyanto, D., Carman, O., Sulistyowati, D.T., et al. (2022). Analysis of combining ability and heterotic estimation on a complete Diallel cross involving five strains of common carp (*Cyprinus carpio*) from different geographical regions in West Java, Indonesia. *Aquac Res*. 53(18):6900-6909. doi:10.1111/are.16155
- Brito, L.F., Kijas, J.W., Ventura, R.V., Sargolzaei, M., Porto-Neto, L.R., Canovas, A., Feng, Z., Jafarikia, M., Schenkel, F.S. (2017). Genetic diversity and signatures of selection in various goat breeds revealed by genome-wide SNP markers. *BMC Genomics* 18 (1), 229.
- Gao F, Li P, Yin Y, et al. (2023). Molecular breeding of livestock for disease resistance. *Virology*. ;587(July):109862. doi:10.1016/j.virol.2023.109862
- Himawan, Y., Alimuddin, Nirmala, K., Imron, Suharyanto. (2022). Ventilation frequency and co1 gene expression in synthetic population of common carp (*Cyprinus carpio*) under high ammonia conditions. *J Med Aquaculture*. Vol 17 (1):1-7.
- Himawan, Y., Syahputra, K., Ariyanto, D. (2017). Growth performance of third-generation Rajadanu common carp (*Cyprinus carpio*) selected through "walkback" method. *J Res Aquaculture*. 12(2):121. i:10.15578/jra.12.2.2017.121-129.
- Lee, C.J., Paull, G.C., Tyler, C.R. (2018). Effects of environmental enrichment on survivorship, growth, sex ratio and behaviour in laboratory Maintained zebrafish *Danio rerio*. *Journal of Fish Biology*. 13865.
- Monaghan, S.J., Chee, D., Adams, A., et al. (2022). Serological analysis of historical field samples reveals major inconsistency between PCR and antibody ELISA for establishing KHV infection status of groups and individual koi. *Aquaculture*. 546:737336. doi:10.1016/j.aquaculture.2021.737336
- Nhan, D.T., (2020). Effects of stocking density on growth performance, survival rate and economic efficiency of Asian seabass (*Lates calcarifer*) cultured in earthen pond. *Journal Agriculture and Development*. 19, 62-70.
- Nhan, D.T., Tu, N.P.C.T., Tu, N.V. (2022). Comparison of growth performance, survival rate and economic efficiency of Asian seabass (*Lates calcarifer*) intensively cultured in earthen ponds with high densities, *Aquaculture*,554: 738151.
- Rakus, K.L., Wiegertjes, G.F., Adamek, M., Bekh, V., Stet, R.J.M., & Irnazarow, I. (2008). Application of PCR-RF-SSCP to study major histocompatibility class II B polymorphism in common carp (*Cyprinus carpio* L.). *Fish & Shellfish Immunology*. 24:734-744.
- Song, H., Zhu, B., Dong, T., Wang, W., Hu, M., Yan, X., Xu, S., Hu, H. (2023). Whole genome resequencing reveals selection signatures for caviar yield in Russian sturgeon (*Acipenser gueldenstaedtii*). *Aquaculture* 568.
- Syahputra, K., Palimirmo, S.P., Himawan, Y., & Ariyanto, D. (2016, April). Performance of KHV resistance in Rajadanu common carp (*Cyprinus carpio*) selected using the molecular marker Cyca-DAB1*05. *Proceedings of the Aquaculture Technology Innovation Forum*. Fisheries Research and Development Center.hlm. Surabaya. 919-924.
- Tave, D. (1993). *Genetic for fish hatchery managers*. 2nd ed. Connecticut: AVI. Publishing Company Inc., 418 pp.

Zhang, Z., Xu, X., Wang, Y., Zhang, X. (2020). Effects of environmental enrichment on growth performance, aggressive behavior and stress-induced changes in cortisol release and neurogenesis of black rockfish *Sebastes schlegelii*. *Aquaculture*. 528, 735483.