The effect of natural plants on the egg yolk absorption rate of Asian redtail catfish (*Hemibagrus nemurus*)

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Abstract. Asian redtail catfish (*Hemibagrus nemurus*) is a freshwater fish commodity has high economic value and is very popular. However, the supply of fry is very limited both in terms of quality and quantity. The purpose of this research was to analyze the use of different types of natural plant extracts on the absorption rate of egg yolk of *H. nemurus* fish larvae. This research was conducted from June to July 2023 at the Fish Hatchery and Breeding Laboratory, Faculty of Fisheries and Marine, University of Riau, Indonesia. The study used was a completely randomized design with immersion treatment using several different types of natural plants. The treatments consisted of: P0 - control treatment without immersion in natural solution; P1 - immersion treatment with pineapple (*Ananas comosus*) solution dose of 1 mL L^{-1} of water; P2 - immersion treatment with wuluh starfruit (*Averrhoa bilimbi*) solution dose of 4 mL L^{-1} of water; P3 - immersion treatment with cherry (*Mutingia calabura*) leaf solution dose of 4 mL L^{-1} of water. The results of the research showed that the best treatment was P3, with an egg yolk absorption time of 120 h after the egg hatched; the percentage of egg yolk absorption rate was 8.84%, the growth rate of the length of *H. nemurus* larvae was 0.65%, the efficiency of utilization of *H. nemurus* egg yolk was 7.305%.

Key Words: cherry leaves, egg yolk utilization efficiency, length growth rate, pineapple, wuluh starfruit.

Introduction. Asian redtail catfish (*Hemibagrus nemurus*) is one of the freshwater fish commodities in Riau Province, Indonesia. This fish is included in the "catfish" group, being one of the 31 economically important species of fish in the waters of the Kampar River, Riau (Sukendi 2014). *H. nemurus* rearing business has grown rapidly. The success of *H. nemurus* cultivation is largely determined by the quality of the fry being cultivated, where the provision of fry for cultivation can be done through artificial spawning (Sukendi et al 2022b). Moreover, the availability of sufficient fry, both in quality and quantity, is a factor that determines the success of a cultivation business (Sukendi et al 2020). However, the success of artificial spawning is very dependent on the quality of the eggs produced from the prospective broodstock, because good quality eggs will produce good quality fry in large quantities (Sukendi et al 2016). Cultivation activities are a solution for increasing fishing yields (Sukendi et al 2021). The problem encountered in hatching *H. nemurus* eggs is the inherent nature of the eggs, which affects the hatchability. According to Indra et al (2014), fish eggs that stick and clump together can prevent oxygen from entering the eggs, and may die. To handle the problems mentioned, the eggs can be immersed in solutions with several types of natural plants that can eliminate the stickiness. Some plants that can be used are pineapple (*Ananas comosus*), wuluh starfruit (*Averrhoa bilimbi*), and cherry leaves (*Mutingia calabura*).

In addition, the problem encountered after the hatching of *H. nemurus* eggs is the large number of early deaths in the larvae phase. This happens when the yolk runs out, and the fish have not been able to find suitable food. External feeding should be carried out when the egg yolk is exhausted, so that the larvae have sufficient nutrition (Mariska et al 2013). Thus, the aim of this paper is to find the best plant species for the absorption rate of *H. nemurus* fish egg yolk, so that fry production in hatcheries can be increased and obstacles to the early phase death of *H. nemurus* can be overcome.

Material and Method. This research was conducted from June to July 2023 at the Fish Hatchery and Breeding Laboratory, Faculty of Fisheries and Marine, University of Riau, Indonesia. The treatments in this study consisted of: P0 - control without immersion in natural ingredients solution; P1 - immersion with pineapple solution 1 mL L^{-1} water; P2 - immersion with wuluh starfruit solution 4 mL L^{-1} water; P3 - immersion treatment with cherry leaf solution 4 mL L^{-1} of water. The design used was a completely randomized random design with 4 levels of treatment and 3 repetitions (12 units of treatment).

The production of the pineapple solution was carried out using pineapple from Rimbo Panjang, Pekanbaru city, bought in bulk at 300 g. Pineapples were smoothed with scratches, the resulting matter being scrubbed and filtered using filtering fabric. The filtration produced a solution of pineapple ready to be used as a treatment. The preparation of the wuluh starfruit solution was done by cleaning wuluh starfruit from Pekanbaru. 4 g of wuluh starfruit were smoothed up and boiled in 1 L of water. After boiling, the decoction water was cooled and filtered. The solution of wuluh starfruit was ready to be used as a treatment. The cherry leaf extract was produced by drying and smoothing the leaves using a blender to obtain the powder. The powder was submerged in a 96% ethanol solution of 3 L until the entire powder of the cherry leaf was soaked, and left for 48 h. The solution was evaporated using a rotary evaporator at 60°C to get a thick solution. The cherry leaf extract was inserted into a closed glass bottle and was ready for use.

The fish eggs were obtained from the artificial breeding of the two pairs of broodstock fish at the Fish Hatchery and Breeding Laboratory, Faculty of Fisheries and Marine Sciences, Riau University, Pekanbaru. Before the injection, the body weight of the broodstock fish was determined to correctly dose the ovaprim used. The dosage used for the injection of the females was 0.5 mL kg^{-1} body weight and for males it was 0.3 mL kg^{-1} body weight. The first female had a body weight of 310 g, and the second female weighed 400 g. The first male weighed 200 g, the second male 300 g. The injection was performed twice within a six-hour interval. The female stripping was done 12 h after the second injection, and the male underwent a surgical procedure to extract the sperm. The fertilization process was carried out by mixing the eggs and sperm. Immersion of the fertilized eggs was carried out for 4 minutes in a basin with a diameter of 35 cm, containing coconut milk filter, with a volume of 1 L for each treatment with a predetermined dose. 1 g of eggs were soaked in each treatment and then they were transferred to the incubation container with 12 units of aquariums measuring 30x30x30 cm^3 to observe the measured parameters. The water quality parameters determined were pH, temperature and dissolved oxygen.

The parameters observed and measured in this study are the following: egg yolk absorption time, egg yolk absorption rate, percentage of reduction in egg yolk volume, egg yolk absorption, larvae growth rate, efficiency of egg yolk utilization, and survival rate.

The egg yolk absorption rate was determined by calculating the egg yolk volume using the formula (Hemming & Buddlington 1988):

$$V = 0.1667\ \pi\ LH^2$$

Where: V - volume of the egg yolk (mm^3); L - the elongated diameter of the egg yolk (mm); H - the shorter diameter of the egg yolk (mm).

The percentage of reduction in egg yolk volume was determined using the formula (Hemming & Buddlington 1988):

$$\text{Reduction} = \left(\frac{Vt - Vo}{Vo}\right) x\ 100$$

Where: Vt - egg yolk volume to -t (mm^3); Vo - egg yolk volume at the beginning (mm^3).

Egg yolk absorption was determined using the following formula (Kohno et al 1986):

$$-g = \left(\frac{1}{t} Ln \frac{Vt}{Vo}\right) \times 100$$

Where: - shows a decline; g - egg yolk absorption (%); Vt - egg yolk volume to -t (mm^3); Vo - egg yolk volume at the beginning (mm^3); t - time required (hours).

The growth rate of larvae length was determined using the following formula (Ricker 1979):

$$\alpha = \left(\frac{LnLt - LnLo}{t}\right) \times 100$$

Where: α - growth rate in length (%); Lt - average length of fish at the end of observation (mm); Lo - average length of fish at the beginning of observation (mm); t - time (hours).

Efficiency of egg yolk utilization was determined using the following formula (Ryland & Nichols 1967):

$$EP = \frac{\alpha}{g} \times 100\%$$

Where: EP - efficiency of egg yolk utilization (%); α - growth rate in length (%); g - yolk absorption rate (%).

The survival rate was determined using the following formula (Effendie 2002):

SR = Nt/No x 100

Where: SR - survival rate (%); Nt - number of larvae at the end of the study; No - number of larvae at the beginning of the study.

The data obtained was tabulated and statistical tests were carried out using the SPSS 22 application. The statistical tests carried out were homogeneity of variances and one-way ANOVA. If the results of the ANOVA test showed a significant difference ($p<0.05$), the SNK test was further employed to determine differences between treatments.

Results and Discussion

Egg yolk absorption time. The results of observations on the absorption time of the egg yolk of *H. nemurus* from each treatment can be seen in Figure 1.

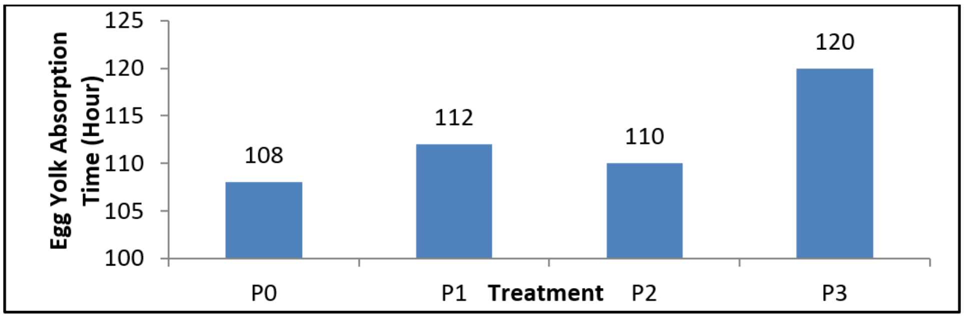

Figure 1. Histogram of egg yolk absorption time of *Hemibagrus nemurus* larvae from each treatment; P0 - control treatment without immersion in natural ingredients solution; P1 - immersion treatment with pineapple solution of 1 mL L^{-1} of water; P2 - immersion treatment with wuluh starfruit solution of 4 mL L^{-1} of water; P3 - immersion treatment with cherry leaf solution of 4 mL L^{-1} of water.

According to Figure 1, the best treatment for *H. nemurus* fish larvae egg yolk absorption was treatment P3, with 120 hours. In the endogenous feeding phase, fish larvae use egg yolk as a nutrient to meet energy needs used in metabolic processes. The egg yolk is used for growth and development.

Egg immersion treatment with different types of natural plants had a significant effect ($p<0.05$) on the egg yolk absorption time. Further test results showed that all treatments had a significantly different effect ($p<0.05$).

Egg yolk absorption rate. The results of observations on the rate of egg yolk reduction from each given treatment can be seen in Figure 2.

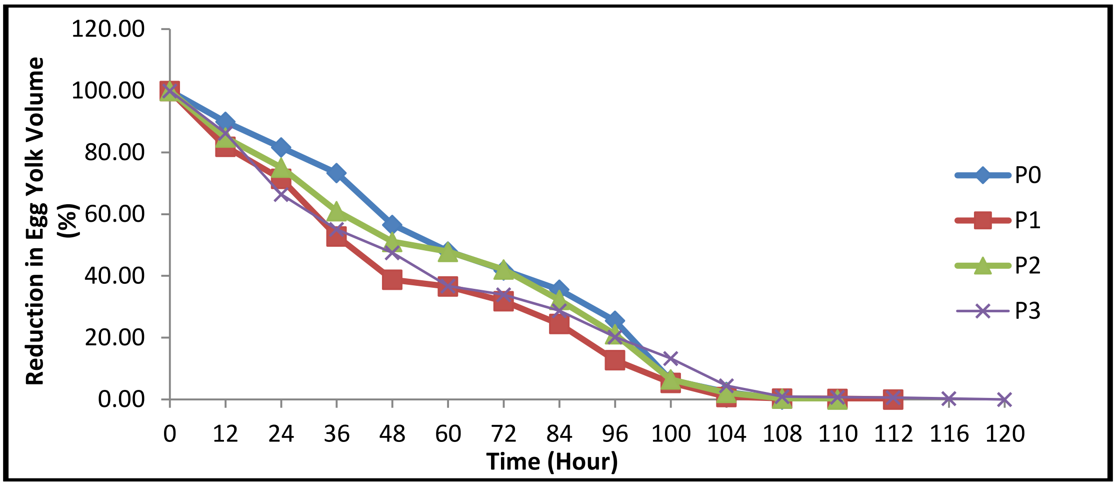

Figure 2. Depreciation of the yolk volume of *Hemibagrus nemurus* larvae.

The egg yolk volume of *H. nemurus* larvae decreased more quickly in the control treatment. Shrinkage in egg yolk volume decreased rapidly at the beginning of absorption until the 100th hour, then absorption slowed down until the yolk was completely absorbed. The reduction in egg yolk volume in P0 at the 104th hour was 97.55%, then it reached 100% at the 108th hour after hatching. Meanwhile, in treatment P1, the percentage reduction in egg yolk volume at the 104th hour was 99.18%, then it reached 100% at the 112th hour. The reduction in egg yolk volume in P2 at the 12th hour was 15.12%, at the 72nd hour it was 57.91%, and it then slowed down until 100% at the 110th hour after hatching. In P3, the percentage reduction in egg yolk volume at the 12th hour was 13.17%, at the 96th hour it was 79.74%, and it reached 100% at the 120th hour after hatching. The volume of the yolk of fish larvae decreases with time, with the development of organogenesis and with increase in length (Pramono & Marnani 2009). The results of observations of the rate of egg yolk absorption from each treatment given can be seen in Figure 3.

P0 showed the highest rate of absorption of the egg yolk with 9.89%, followed by P2 with 9.65%, P1 with 9.4%, and P3 with 8.84%. The results of the first observation showed that newly hatched *H. nemurus* larvae were transparent and had an average egg yolk volume of 0.139 mm^3. The rate of absorption of egg yolk based on volume showed differences in each treatment. The egg immersion treatment with different types of natural plants provided had a significant effect ($p<0.05$) on the egg yolk absorption time of *H. nemurus* larvae. Further test results using the Student-Newman-Keuls test showed that all treatments had a significantly different effect ($p<0.05$).

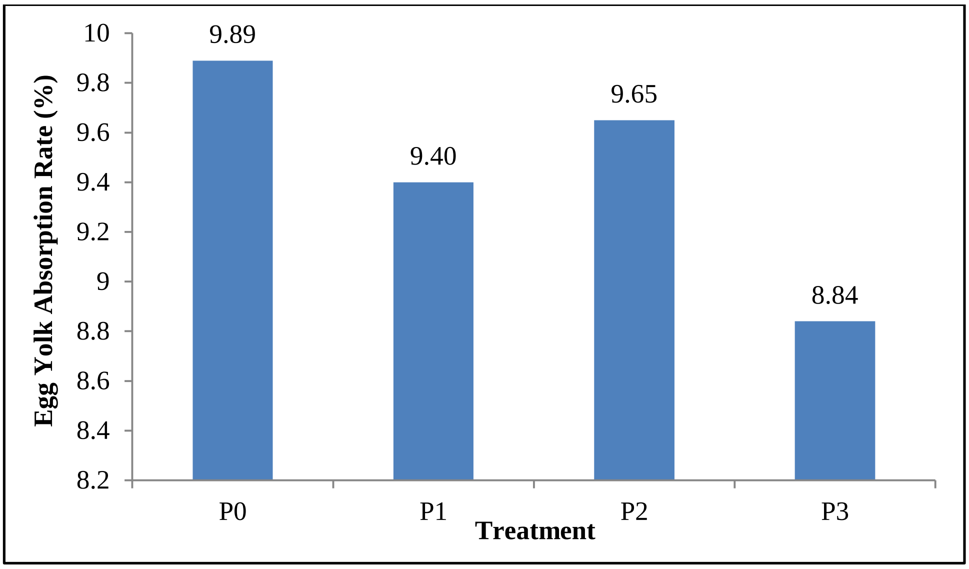

Figure 3. Histogram of the yolk absorption rate of *Hemibagrus nemurus* larvae.

Length growth rate. The newly hatched *H. nemurus* larvae had an average total length of 4 mm. The final length of *H. nemurus* larvae during observation showed differences in each treatment. The average length during observation for each treatment given can be seen in Figure 4.

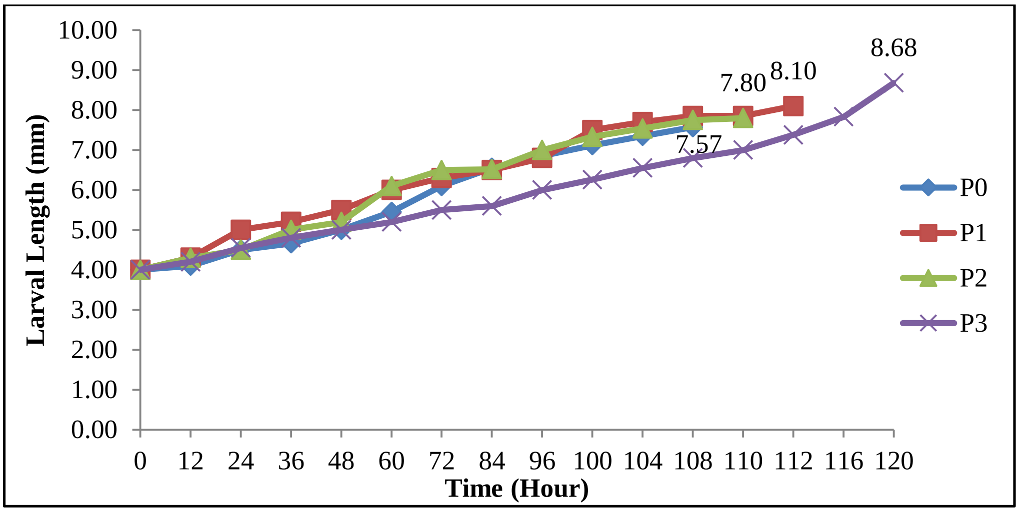

Figure 4. Average length of *Hemibagrus nemurus* larvae.

The growth in length of *H. nemurus* increased at each observation and produced a sigmoid curve, where the growth of the larvae in the early phase of its life is slow then fast and slow again when the yolk runs out. According to Nuswantoro et al (2019), the reduced volume of egg yolk is offset by an increase in the length of the larvae; the absorbed egg yolk is used for growth, repair of damaged cells and tissues in the body. The results of observations of the length growth rate in each treatment can be seen in Figure 5.

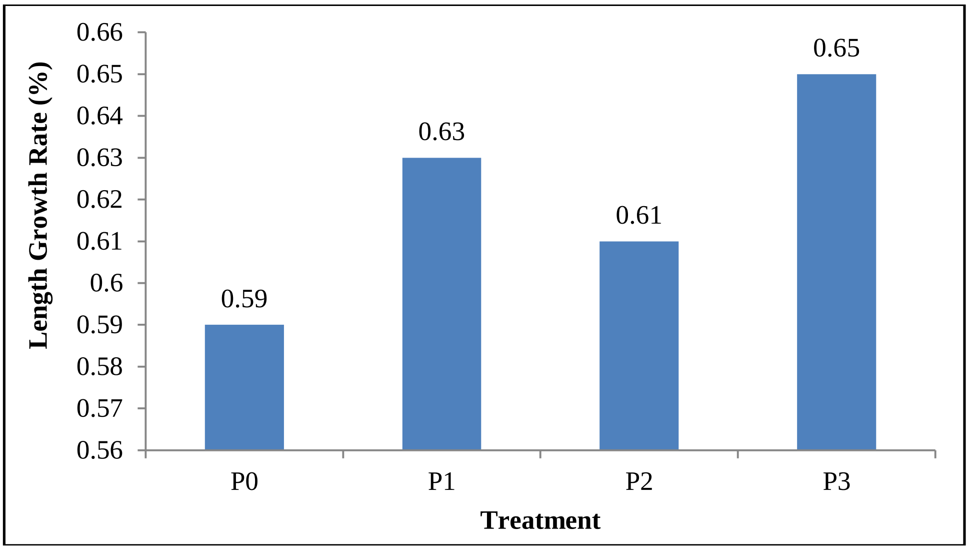

Figure 5. Growth rate of *Hemibagrus nemurus* larvae.

The highest rate of absorption of the egg yolk absorption of *H. nemurus* larvae sequentially was in P3 with 0.65%, P1 with 0.63%, P2 with 0.61%, and P0 with 0.59%. P0 had the lowest length growth rate. The growth rate of the length of the larvae during the observation was inversely proportional to the rate of absorption of the yolk. According to Nugraha et al (2012), high metabolic rate causes faster absorption of egg yolk. Thus, the growth is greatly influenced by the amount of energy lost during this development period (Hariyandi et al 2020).

The egg immersion treatments with different types of natural plants had a significant effect ($p<0.05$) on the egg yolk absorption time of *H. nemurus* larvae. Further test results using the Student-Newman-Keuls test showed that all treatments had a significantly different effect ($p<0.05$).

Efficiency of egg yolk utilization. The results of observations on the rate of shrinkage of the yolk from each of the treatments is presented in Figure 6. The treatments with the greatest efficiency in utilizing *H. nemurus* larvae egg yolk in sequence were: P3 with 7.305%, P1 with 6.701%, P2 with 6.288%, and P0 with 5.973%. Treatments with a lower length growth rate resulted in a low efficiency value for egg yolk utilization. P0 resulted in a low utilization efficiency of the yolk because the energy generated from the metabolism of the yolk was not only used for the growth and activity of the larvae, but also to maintain survival (Yahya et al 2015).

According to Mulyani et al (2015), the efficiency of egg yolk utilization is the amount or size of body tissue formed from the absorption of egg yolk. The egg yolk is used to maintain and develop body organs until the larvae begin to actively swim. The rapid increase in larval length depends on the rate of absorption of the egg yolk (Dharma 2015). The egg yolk in the larvae undergoes a process of absorption, transport and metabolism. Not all the nutrients from egg yolk are absorbed and are used for the organogenesis process.

The treatments of egg immersion in different natural plant solutions had a significant effect ($p<0.05$) on the efficiency of utilizing the egg yolk. Further test results using the Student-Newman-Keuls test showed that all treatments had a significantly different effect ($p<0.05$).

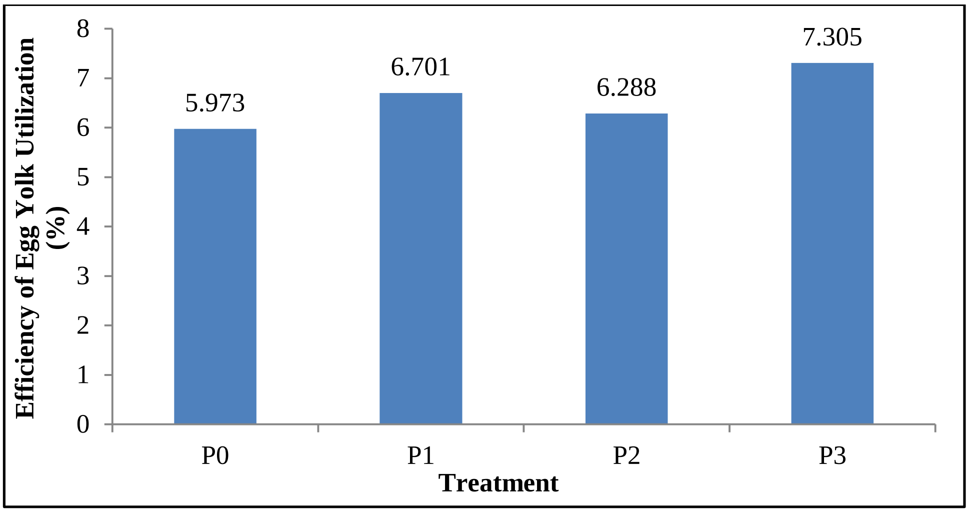

Figure 6. Average egg yolk utilization efficiency of *Hemibagrus nemurus* larvae.

Survival rate. The survival rate of *H. nemurus* larvae from each treatment are presented in Figure 7.

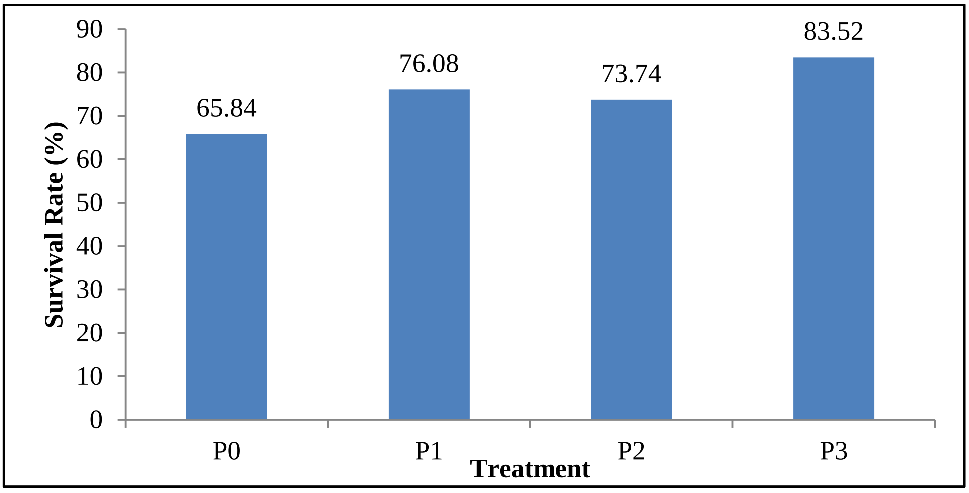

Figure 7. Survival of *Hemibagrus nemurus* larvae after 42 days of rearing after egg immersion in different plant solutions.

The treatments that produced the highest survival value in sequence were: P3 with 83.25%, P1 with 76.08%, P2 with 73.74% and P0 with 65.84%. Sukendi et al (2022a) showed that the survival of *H. nemurus* larvae which hatched from eggs immersed in pineapple solution was 71.97%, while the survival of *H. nemurus* larvae from eggs immersed in cherry leaf solution was 86.67% (Sukendi et al 2023). According to Mahardika et al (2017), survival is influenced by two factors, namely biotic factors and abiotic factors. The survival rate of larvae after hatching is also influenced by the quality of the eggs produced by parent fish (Saputra et al 2013).

The egg immersion treatments in different types of natural plant solutions had a significant effect ($p<0.05$) on the survival of *H. nemurus* larvae. Survival rates in P0 and P3 were significantly different than survival rates in P2 and P1 ($p<0.05$), while the survival rate in P2 was not significantly different to that of P1.

Conclusions. The use of different types of natural plants affects the absorption time of egg yolk, the rate of egg yolk absorption, the growth rate of length and the efficiency of egg yolk utilization. The best natural plant treatment in this study was immersion in a solution of cherry leaf extract using a dose of 4 mL L^{-1} of water, resulting in an egg yolk absorption time of 120 h after the egg hatched, the percentage of egg yolk absorption rate was 8.84%, and the efficiency of utilizing egg yolk was 7.305%.

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Conflict of Interest. The authors declare that there is no conflict of interest.

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