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The effect of soaking time of carp (*Cyprinus carpio*) eggs in starfruit (*Averrhoa bilimbi*) extract on adhesiveness and hatchability

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Abstract
The purpose of this research was to determine the effect of the duration of soaking carp eggs in starfruit extract on the adhesiveness and hatching rate of the eggs. The production of carp seeds in the aquaculture sector has a limited system, thick mucus on carp eggs can affect the hatchability rate. Mucus that is too thick will cause the eggs to clump, thus inhibiting embryo development. Tannins can bind proteins in carp egg mucus glycoproteins which can reduce adhesiveness. Different time for soaking carp eggs in starfruit extract are needed to find out whether glycoproteins will accumulate along with the length of soaking. The number of treatment containers was 12 using jars with a volume of 10 liters. There were 4 treatment levels and 3 replications. The treatments tested were the length of time for soaking carp eggs in starfruit extract for A (0 minutes), B (5 minutes), C (10 minutes), and D (15 minutes). The extract dose used was 4 ml/L. The results of the research that has been carried out show that soaking carp eggs for 15 minutes produces the lowest adhesive power of 54.00% and the highest hatching power of 86.00%.

Keywords: Adhesiveness, carp, hatching rate, starfruit

Introduction
Carp (*Cyprinus carpio*) is a freshwater fish widely cultivated in Indonesia. The choice of carp as a cultured commodity was driven by high consumption demand. According to data, the average per capita consumption of carp in Indonesia is quite high, at 922.7 kg per week (Central Statistics Agency, 2024). The hatchery process is crucial in the fisheries sector, providing fish fry for further growth. Carp naturally live in rivers with strong currents and spawn naturally. Natural spawning can be done simply, but the success rate is quite low. This can be caused by the quality of the broodstock, environmental conditions, and the availability of natural food (Ritonga *et al*., 2024) [16]. The mucus on carp eggs serves to protect the eggs and act as a medium for attachment during natural spawning. Carp seed production in aquaculture is limited, and thick mucus on carp eggs can affect hatchability. Excessively thick mucus on fish eggs can cause the eggs to clump together, preventing the embryo from developing properly. The glycoprotein layer found in carp egg mucus can cause eggs to stick together (Sahi *et al*., 2023) [18]. Reducing this glycoprotein layer can help reduce the percentage of eggs that stick together. One natural ingredient that can reduce the adhesiveness of carp eggs is starfruit. Starfruit contains tannins, sulfur, formic acid, and potassium citrate, where the tannin content in starfruit can increase hatchability (Malik and Inriyani, 2015) [8]. One compound that can reduce egg adhesion is tannin. According to research by Yohanes *et al*. (2022) [23], catfish eggs soaked in a tannin solution produced the lowest adhesion of 1.00% and the highest adhesion of 2.13%. The duration of soaking fish eggs can affect the degree of fertilization and hatching (Pratiwi *et al*., 2020) [13]. Excessive soaking times and inappropriate extract dosages can risk egg damage, can be toxic, and can cause embryo death (Putri and Kurniawan, 2025) [15]. Different soaking times for carp eggs in starfruit extract are necessary to determine whether glycoproteins will accumulate with the duration of soaking.

Material and Methods
The equipment and materials used in this study included one male carp (*C. carpio*), approximately 2 year old, weighing 2,5 kg, and one females, also around 1 year old, weighing

1,7 kg all of which were healthy. These fish were obtained from Balai Benih Ikan Mijen, Semarang. The eggs were collected through artificial spawning, with 50 eggs used per treatment. *Salmon Gonadotropin Releasing Hormone*, under the brand "Ovaspec," were used for final maturation and egg release in the carp, while tannin was used to reduce the adhesiveness of the carp eggs. The tannin used was from starfruit extract. The research method used was an experimental approach. The experimental design employed in this study was a completely randomized design (CRD) with 4 treatments and 3 replications. Each replication consisted of 50 eggs, totaling 600 eggs. The dose of starfruit extract used in this study refers to the research of Putri *et al*. (2022) [14], which is 4.0 ml/L. The carp larvae were reared for 7 days. They were fed live silkworms (*Tubifex* sp.) ad libitum. The silkworms were fed to the carp larvae 2 days after hatching.

The soaking times used in this study refer to the research by Amalia *et al*. (2023) [1], as follows:

- **Treatment A:** Soaking eegs in starfruit extract for 0 minutes
- **Treatment B:** Soaking eegs in starfruit extract for 5 minutes
- **Treatment C:** Soaking eggs in starfruit extract for 10 minutes
- **Treatment D:** Soaking eggs in starfruit extract for 15 minutes

Container preparation

The test containers used included a basin for soaking eggs and a jar for hatching. The basin with a diameter of 20 cm and jar with a volume of 10 liters were cleaned and washed using clean water. The basin and jar were soaked in a Potassium Permanganate solution for approximately 1 hour. The basin and jar were rinsed using water until clean. The test containers were arranged according to the design that had been made. All clean test containers were filled with 1 liter of water. Aeration and a heater were installed in each jar, then a round filter was placed on top of the jar so that it touched the water.

Making starfruit extract

The extract of starfruit was prepared by weighing 4 grams of starfruit. The starfruit was washed with water until clean. The starfruit was then sliced into small pieces. 4 grams of starfruit were boiled in 1 liter of distilled water. Wait until it boils and the distilled water reduces, then the starfruit was removed and the boiled water was cooled. The boiled water was filtered using a sieve. The pH of the starfruit extract was measured first using a pH meter before being used in the study. The starfruit extract can be stored in an airtight container such as a bottle and can be stored in the refrigerator.

Carp spawning

The fish spawning technique used in this study is artificial spawning of carp broodstock with hormonal stimulation by injecting Salmon Gonadotropin Releasing Hormone (GnRH). Hormone injection is done at night. Male and female broodstock are placed in a basin, then the broodstock are weighed to determine their body weight so that the hormone dosage can be calculated. Hormone injection is done on male broodstock with a hormone dose of 0.5 ml/kg body weight and on female broodstock with a hormone dose of 0.6 ml/kg body weight. The spawning process will be carried out in the morning after approximately 9 hours of hormone injection. The female broodstock is stripped on the abdomen and massaged until the eggs come out. Stripping is done on the male broodstock on the abdomen and massaged until the sperm comes out, then the sperm is diluted with NaCl solution. The sperm that has been diluted with NaCl solution is then mixed with the eggs and stirred evenly using a chicken feather. After the sperm and eggs are evenly mixed, the eggs are rinsed using clean water slowly to remove any remaining sperm.

Soaking eggs

The treatment of soaking carp eggs in starfruit extract used in this study was by soaking 50 carp eggs in each egg soaking container in the form of a basin. 4.0 ml of starfruit extract was added to each basin that had been filled with 1 liter of clean water. 50 carp eggs were soaked in each basin for 0 minutes, 5 minutes, 10 minutes, and 15 minutes. The soaking of carp eggs was done by stirring the eggs using a chicken feather in the extract soak slowly. After the soaking time was over, the soaking water was slowly drained and the eggs were rinsed using clean water to remove the remaining extract soak. The cleaned eggs were placed in a jar for hatching.

Larvae Maintenance

Carp larvae were reared for 7 days. Live silkworms (*Tubifex* sp.) were fed ad libitum. The silkworms were fed to the carp larvae 2 days after hatching. Water quality parameters were measured in the egg hatching tank and the larval rearing tank. Water quality in the egg hatching tank was measured before the eggs were placed in the hatching tank. Water quality in the larval rearing tank was measured daily at 8:00 AM and 4:00 PM.

Adhesiveness

The research variable used is adhesive power, which can be calculated using a formula based on research by Mulyani and Johan (2020) [10], as follows:

$$\text{Adhesiveness} = \frac{\text{number of eggs attached}}{\text{total number of eggs}} \times 100\%$$

Description: The criteria for eggs that stick together are eggs that stick together or pile up with each other.

Embryonic development

The development stage of carp embryos will be observed using a microscope with a magnification of 40× and the first observation will be carried out after 30 minutes of fertilized eggs for 5 repetitions, continued every hour for 5 repetitions, and every 2 hours until hatching.

Hatching time

The hatching time can be calculated using a formula based on research by Ulyana *et al*. (2018) [22], as follows:

$T = T_n - T_0$

Description: T = Egg hatching time (hour)

T_n = Egg hatching time (hours)

T_0 = Time to fertilization (hours)

Hatching rate

Hatchability can be calculated using a formula based on research by Ula *et al*. (2023) [21], as follows:

$$HR = \frac{\text{number of eggs hatched}}{\text{total number of eggs}} \times 100\%$$

Survival rate

Survival Rate can be calculated using the formula on research by Dinda and Aminullah (2024) [3], as follows:

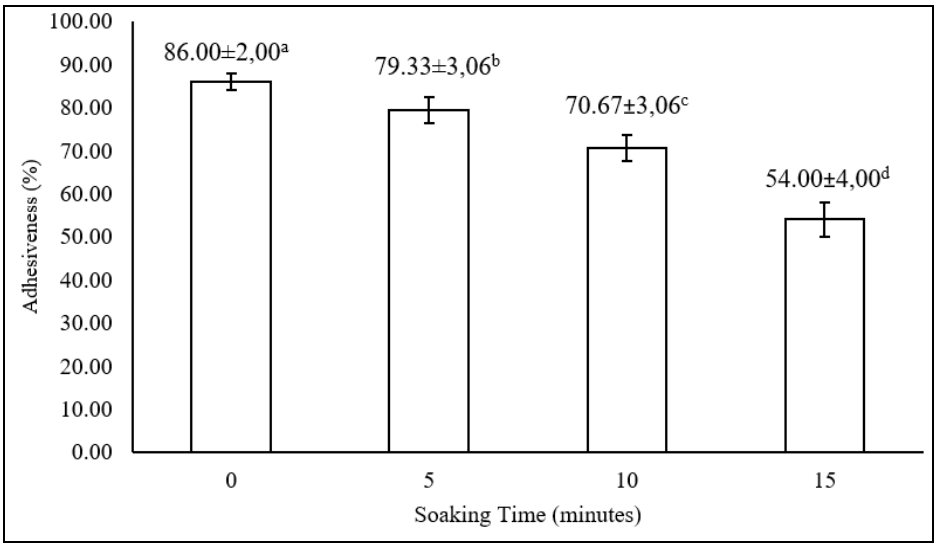
$$SR = \frac{Nt}{N0} \times 100\%$$

Description: SR = *Survival rate* (%)

N_t = Number of carp larvae at the end of the study (units)

N_0 = Number of carp larvae at the beginning of the study (units)

Hatching rate



Water quality

Water quality measurement parameters include temperature, pH, and dissolved oxygen in the egg hatching and larval rearing containers. Water quality in the egg hatching containers is measured before the eggs are placed in the hatching containers. Water quality in the larval rearing containers will be measured daily at 8:00 a.m. and 4:00 p.m. WIB for 7 days. These water quality measurements are taken using several tools, including a DO meter and a pH meter.

Result and Discussion

Adhesiveness

Based on the results of research that has been conducted regarding the effect of the length of soaking of carp (*C. carpio*) eggs in extract of starfruit (*A. bilimbi*) on adhesive power and hatchability, the results of the calculation of egg adhesive power are presented in Figure 1.

Based on the histogram above, it can be seen that the results of the adhesion of carp (*C. carpio*) eggs show the highest average adhesion results in treatment A with egg immersion for 0 minutes of 86.00±2.00%. The results of the calculation of the lowest average adhesion results in treatment D with egg immersion for 15 minutes of 54.00±4.00%.

Embryonic stages

Based on the results of research that has been conducted regarding the effect of the length of soaking of carp (*C. carpio*) eggs in extract of starfruit (*A. bilimbi*) on adhesive power and hatchability, the stages of development of carp embryos are presented in Figure 2.

Based on the results of observations of the development stages of goldfish embryos in the research that has been carried out in all treatments, they experience the cleavage stage, morula stage, blastula stage, gastrula stage, organogenesis, and the embryo will hatch into a larva.

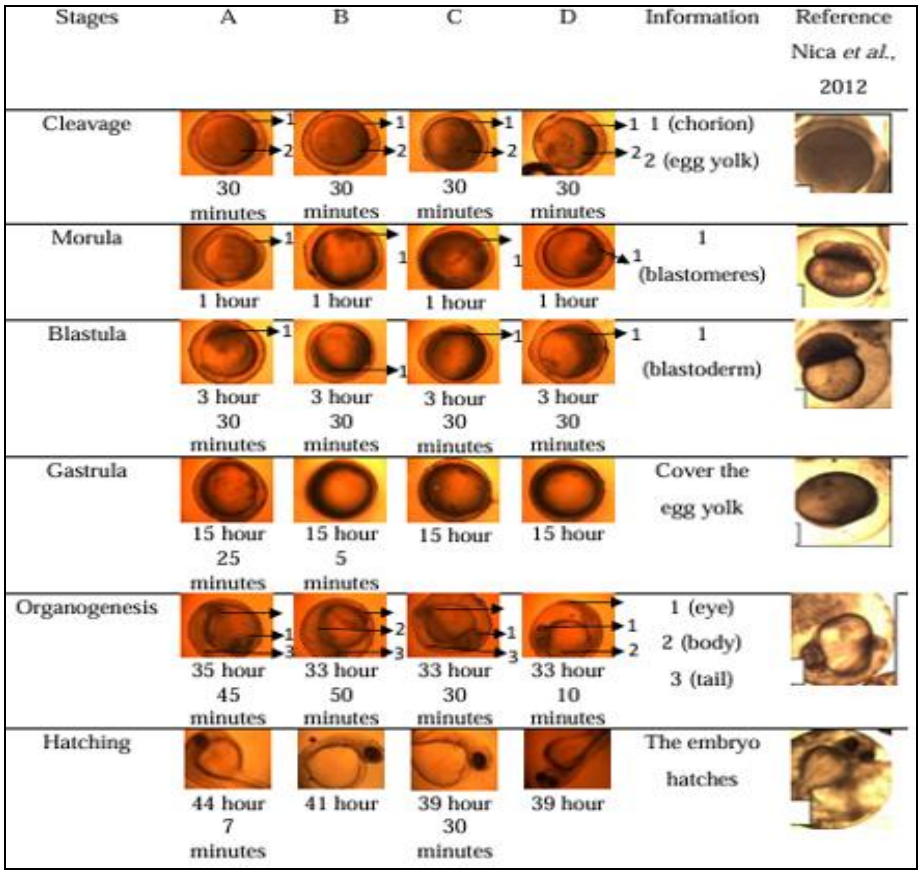
Hatching time

Based on the results of research conducted on the effect of soaking time for carp (*C. carpio*) eggs in starfruit (*A. bilimbi*) extract on adhesiveness and hatchability, the hatching time for carp eggs is presented in Table 1.

Table 1: Hatching time

	Treatment			
Hatching Time (hours)	**A (0 minutes)**	**B (5 minutes)**	**C (10 minutes)**	**D (15 minutes)**
	44 hours 7 minutes	41 hours	39 hours 30 minutes	39 hours

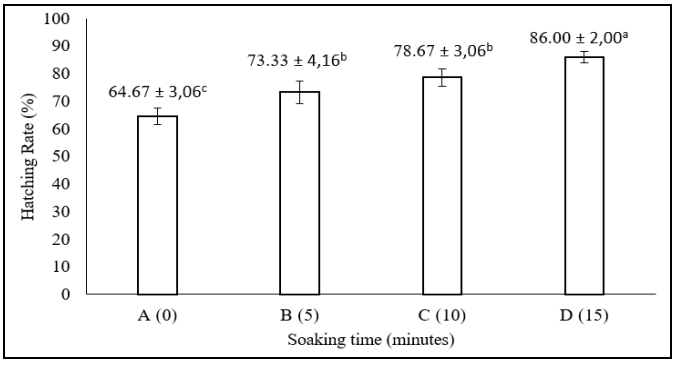
Based on the results obtained, it shows that the hatching time of carp eggs in the four treatments is different. It can be seen that carp eggs soaked in starfruit extract for 15 minutes showed the fastest hatching time, while carp eggs soaked for 0 minutes showed the longest hatching time. Based on these results, it can be seen that fish eggs soaked in starfruit extract for 15 minutes can hatch 4 hours 53 minutes faster than eggs soaked for 0 minutes.



Hatching rate

Based on the results of research that has been conducted regarding the effect of the length of soaking of carp (*C. carpio*) eggs in extract of starfruit (*A. bilimbi*) on adhesive power and hatchability, the results of the calculation of egg hatchability are presented in Figure 3.

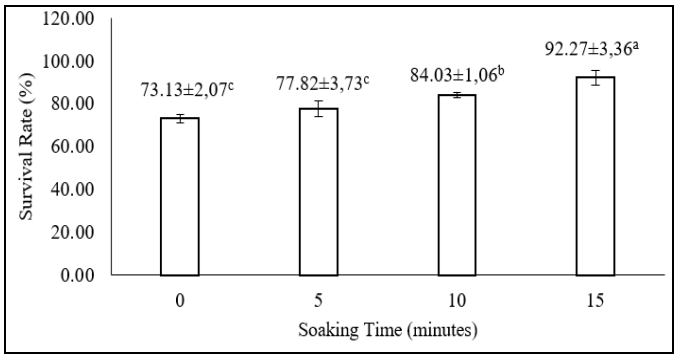
Based on the results of the hatchability test of common carp (*C. carpio*) eggs, treatment A (0-minute egg aimmersion) showed a significant difference compared to treatment B (5-minute egg immersion), treatment C (10-minute egg immersion), and treatment D (15-minute egg immersion). Treatment B (5-minute egg immersion) was not significantly different from treatment C (10-minute egg immersion), but was significantly different from treatment A (0-minute egg immersion) and treatment D (15-minute egg immersion). Soaking common carp eggs in starfruit extract for 15 minutes resulted in a 21.33% increase in hatchability compared to immersion for 0 minutes. Highest hatchability in this study was 86%. This indicates that the duration of soaking carp eggs in starfruit extract affects the hatchability of carp eggs. Based on the results obtained, soaking carp eggs for 15 minutes significantly increased hatchability.



Survival rate

Based on the results of research that has been conducted regarding the effect of the length of soaking of carp (*C. carpio*) eggs in extract of starfruit (*A. bilimbi*) on adhesive power and hatching power, the results of the calculation of the survival rate of carp (*C. carpio*) are presented in Figure 4.

Based on the results of the survival rate test for common carp (*C. carpio*), treatment A (egg immersion for 0 minutes) showed no significant difference from treatment B (egg immersion for 5 minutes), but significantly different from treatment C (egg immersion for 10 minutes) and treatment D (egg immersion for 15 minutes). Soaking eggs in starfruit extract for 15 minutes resulted in the highest survival rate.



Water quality

The results of water quality measurements in the carp egg hatching media can be seen in Table 2.

Based on the results of water quality measurements obtained in Table 2, it shows that the pH value in this study ranged from 7.6 to 7.7. The temperature measurement values obtained in this study ranged from 25.4 to 25.7°C. The DO measurement values obtained in this study ranged from 6,44 to 6,93 mg/L.

Table 2: Water quality

Treatment	Temperature (°C)	DO (mg/L)	pH
A	25.4 - 25.6	6.55 - 6.93	7.6 - 7.7
B	25.5 - 25.7	6.53 - 6.92	7.6 - 7.7
C	25.6 - 25.7	6.45 - 6.78	7.6 - 7.7
D	25.5 - 25.7	6.44 - 6.89	7.6 - 7.7
Optimal Value	25 - 30 °C*	> 5 mg/L*	6.5 - 8.5*
Reference	*SNI: 01-6133-1999*		

The results of measuring the water quality of carp larvae for 7 days after the larvae hatch can be seen in Table 3.

Based on the results of water quality measurements obtained in Table 3, it shows that the pH value in this study ranged from 7,7 to 7,9. The temperature measurement values obtained in this study ranged from 27 to 28,5°C. The DO measurement values obtained in this study ranged from 5,72 to 7,47 mg/L.

Table 3: Water quality

Treatment	Temperature (°C)	DO (mg/L)	pH
A	27.2 - 28.3	6.22 - 7.28	7.7 - 7.9
B	27.7 - 27.9	6.26 - 7.47	7.7 - 7.9
C	27.4 - 28.5	6.19 - 7.30	7.7 - 7.9
D	27 - 28.5	5.72 - 7.36	7.7 - 7.9
Optimal Value	25 - 30 °C*	> 5 mg/L*	6.5 - 8.5*
Reference	*SNI: 01-6133-1999*		

Discussion

Based on the test results of the adhesive power of carp (*C. carpio*) eggs, it shows that the length of time the carp (*C. carpio*) eggs are soaked in extract of starfruit (*A. bilimbi*) is significantly different between treatments. The results of the calculation of the adhesive power of eggs soaked in extract of starfruit decreases with increasing soaking time. The glucoprotein layer can be reduced using materials containing tannin because tannin can reduce glucoprotein (Imran *et al*., 2017) [6]. Soaking fish eggs in extract of starfruit containing tannin can reduce the adhesive properties of fish eggs. The tannin content will bind proteins in egg mucus by precipitating proteins that are mutually bound into tannin-protein complex compounds (Yohanes *et al*., 2022) [23]. Tannin compounds can reduce the adhesive level of fish eggs by eroding proteins from the mucus layer of fish eggs. Tannin compounds are included in polar compounds that can dissolve in water, so they can be used as a medium for soaking fish eggs to reduce the level of adhesive power of carp eggs. The mechanism for reducing glycoproteins in egg mucus can be achieved through interactions with the hydroxyl groups of tannins. Tannins contain hydroxyl groups (-OH), which can form hydrogen bonds with the carboxyl, amide, or hydroxyl groups in the glycoprotein chain.

Based on the observation results of the development stages of carp embryos in the research that has been done in all treatments experienced the cleavage stage, morula stage, blastula stage, gastrula stage, organogenesis, and the embryo will hatch into larvae. The difference in the length of time during the hatching process of carp embryos is quite visible in the four eggs between treatments. In treatment D with a 15-minute soaking, the embryo successfully hatched at 39 hours after the egg was fertilized. This proper metabolism (Suriansyah, 2021) [20]. This healthy metabolism allows the embryos to develop properly and hatch quickly. The tannin content in starfruit has a function as a compound that can reduce adhesiveness which reduces egg mucus (Putri *et al*., 2022) [14]. The reduction in mucus can help the oxygen supply be distributed evenly to the eggs, which can accelerate the hatching of carp eggs. Another factor that can affect the length of egg hatching time is the condition of the egg hatching medium. Tannin content reduces egg stickiness, thereby increasing hatchability by reducing glucoprotein in the egg chorion (Amalia *et al*., 2023) [1].

Tannins can reduce the protein in the egg's chorion layer, which can trigger the chorionase enzyme process, accelerating the softening of the chorion, allowing the eggs to hatch (Pratiwi *et al*., 2020) [13].

Biotic factors include the fish's ability to adapt to their environment and age, while abiotic factors include water quality management, rearing media, and food availability (Pratama *et al*., 2015) [12]. Healthy carp larvae will produce a high survival rate. Based on SNI 6133:1999, the survival rate for carp larvae is 70-80%. In this study, the highest SR value reached 92.27%.

The results of this research indicate that water quality measurements during egg hatching and the rearing of carp larvae remained within optimal levels. Water quality is one of the parameters that supports the survival of cultivated biota. Oxygen is needed by all organisms for respiration, metabolism, or the exchange of substances that generate energy for growth and development. Oxygen is also required for the oxidation of organic and inorganic materials in aerobic processes. This finding is supported by Gemilang *et al*. (2017) [4], that the organism's need for dissolved oxygen varies relatively depending on the type of stage and activity.

Conclusion

1. The duration of soaking carp (*C. carpio*) eggs in starfruit (*A. bilimbi*) extract affects their adhesiveness and hatchability.
2. The best duration of soaking carp (*C. carpio*) eggs in starfruit extract was treatment D (15 minutes soaking), which resulted in an adhesiveness of 54.00% and a hatchability of 86.00%.

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