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Impact of Glucose Addition on The Embryo Development of Climbing Perch (*Anabas testudineus* Bloch)

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ABSTRACT

The addition of glucose to the hatching medium is thought to enhance the embryonic development of climbing perch by providing additional energy, strengthening metabolic stability. This research aims to analyze the impact of glucose addition on the embryonic development of climbing perch in developing efficient, sustainable, and conservation-oriented hatchery technology. The study was conducted in the Ulin River, North Banjar Baru District, with broodstock climbing perch from BBII Karang Intan, aged less than 1.5 years and weighing at least 100 g for females and 50 g for males. Fertilized eggs were then incubated in aquadest incubation media using 20 petri dishes, and the media was given glucose solution according to the treatment, namely 0.2 g/100 mL (A), 0.4 g/100 mL (B), 0.6 g/100 mL (C), and without the addition of glucose as a control (D). The addition of glucose solution to the hatching medium has a positive effect on embryo development. Glucose helps increase the metabolic activity of the embryo, accelerates development, and produces optimal hatching power (100%).

INTRODUCTION

The climbing perch is an economically important fish widely cultivated in Southeast Asia due to its high nutritional value, savory flavor, and strong adaptability to suboptimal water conditions (Maulida, 2023). In South Kalimantan, climbing perch is a cultural and economic commodity for the Banjar community, with market prices reaching IDR 50,000–IDR 120,000 per kilogram. The wild population of climbing perch continues to decline due to overexploitation, pollution, and habitat degradation (Ansyari & Slamat, 2023), making the availability of seeds through hatcheries crucial to supporting the sustainability of fish resources.

The main problem in climbing perch cultivation is the low availability of quality seeds due to low hatching rates and larval survival rates. Mustakim et al. (2009) found that most market demand is still met by wild catches because hatchery technology is not yet optimal. The larval stage is the most critical stage, requiring balanced nutrition and stable environmental conditions (Singh, 2024). Previous research showed that climbing perch larval survival rate was only 55% at a density of 10 individuals/L (Susila, 2016), and improving the nutritional quality of the media, such as the use of Brachionus calyciflorus enriched with HUFA (Singh, 2024), can improve larval quality.

Environmental factors, particularly the quality of the hatching media, significantly determine the success of embryonic and larval development. The swamp waters that serve as climbing perch's natural habitat have a temperature of 27–29°C, oxygen levels of 1.5–3.7 mg/L, and a pH of 3.7–6.4, all of which influence embryogenesis and larval survival (Rukmini et al., 2014). The availability of plankton as natural food in the early stages also influences larval growth and survival. Although spawning technologies such as Ovaprim hormone injections or pituitary extracts have been widely mastered (Marlida, 2008), the hatching and early larval stages remain limiting factors requiring innovation.

An increasingly researched approach is the addition of simple energy sources such as honey and glucose to support embryo viability. Honey, which contains approximately 31% glucose and 30% fructose (USDA), has been shown to increase fertility and hatchability in silver barb (Tawes) by up to 54.58%, and larval survival by 71.34% (Andini et al., 2022). In catfish, the addition of 0.7 mL of honey resulted in a hatching rate of 84.83% and larval survival of 82.05% (Hasan, 2018). Glucose, as a monosaccharide, acts as a rapid energy source, maintains cell metabolism, and supports osmotic stability, thus promoting more targeted embryo development.

Glucose effectively improves fish reproductive performance. Wibowo (2007) found that adding 0.9% glucose to silver barb sperm storage media resulted in optimal fertilization. Glucose stability has also been shown to influence the physiological response of silver barb (Jelawat) to environmental stress (Rizki et al., 2020). From a hormonal and nutritional perspective, the success of climbing perch spawning is influenced by the optimal sGnRHa hormone dose of 0.015 g/g body weight (Mandal et al., 2016) and the broodstock's protein requirement of 39–40% to improve gonad quality (Satheesh et al., 2023).

The addition of glucose to the hatching medium is thought to enhance the embryonic development of climbing perch by providing additional energy, strengthening metabolic stability, and increasing the chances of hatching and larval survival. This innovation is particularly important for South Kalimantan, where demand for climbing perch fry is increasing and natural resources are increasingly limited. This research aims to analyze the impact of glucose addition on the embryonic development of climbing perch in developing efficient, sustainable, and conservation-oriented hatchery technology.

LITERATURE REVIEW

The climbing perch is known as a carnivorous fish with omnivorous tendencies, meaning that under certain conditions, it can adapt its diet to the availability of food sources in its environment. In its natural habitat, the climbing perch eats a variety of organisms, including aquatic insects, surface insects, insect larvae, worms, small crustaceans like marsh shrimp, and small juvenile fish. Furthermore, in environments with low animal food availability, the climbing perch can also utilize plant matter such as algae or detritus as a supplementary energy source. This flexible diet demonstrates the climbing perch's high ecological adaptability to changes in the aquatic environment. The climbing perch also possesses a unique physiological ability: it can survive in low dissolved oxygen conditions through its labyrinth organ, which aids in direct air respiration (Ansyari et al., 2008). This adaptation allows the climbing perch to survive in stagnant, muddy waters, and even in nearly dry swamps during the dry season.

An embryo is the initial stage of organism development resulting from the fertilization process between an egg and a sperm cell. In biology, an embryo refers to the early developmental phase before becoming an individual and developing a complete body structure. Fish embryonic development begins with the cleavage stage, which involves cell division, blastulation, gastrulation, and neurulation (Alfiah, 2019). Embryo development continues with the process of organogenesis, during which the body's organs are formed. Embryo development ends when all organ systems are formed (Soedibya & Pramono, 2018).

1. Cleavage Phase
   The cleavage phase begins with the formation of a blastodisk at the animal pole. Complete blastodisk formation occurs 60 minutes after fertilization. This blastodisk will then divide into many cells. Redha et al., (2014) reported that the formation of a single cell in a kelabau fish embryo takes 31 minutes after fertilization (balstodic).
2. Morula Phase
   The morula is the stage of embryonic development following fertilization and a series of cell divisions (cleavage). Cahyanti et al. (2022) stated that the morula phase of embryonic development in silver barb fish occurs at a temperature of 28°C between the 80th and 120th minutes.
3. Blastulation Phase
   The blastulation phase is the initial stage of embryonic structure formation, with an increasing number of blastomere cells in a fluid-filled cavity called the blastocoel (Agatha et al. 2021).
4. Gastrulation Phase
   Gastrulation is the stage of embryonic development after the blastula, during which a major reorganization of embryonic cells occurs to form three main germ layers: ectoderm, mesoderm, and endoderm. These layers will later develop into various organs and tissues of the fish's body.
5. Organogenesis Phase
   This stage involves the development of the major organs in the embryo's body. Suriansyah (2021), entitled The Effectiveness of Using Incubation Temperature

on the Development of Climbing perch Egg Embryos (Anabas testudineus Bloch), shows the latency of climbing perch egg embryo development in each phase using different incubation temperatures.

METHOD

The research site was the Ulin River in North Banjar Baru District. The broodstock of climbing perch came from the Karang Intan Local Fish Farm (BBII) in Banjar Regency. The broodstock used were less than 1.5 years old, females less than 1 year old, and males less than 1 year old. The broodstock were then weighed to determine their weight. The selected broodstock must weigh at least 100 grams for females and approximately 50 grams for males. Fertilized eggs were incubated in a hatching medium (distilled water) using 20 Petri dishes each, to which glucose solution had been added at the specified dosage:
A = Addition of 0.2 grams of glucose solution/100 mL of distilled water
B = Addition of 0.4 grams of glucose solution/100 mL of distilled water
C = Addition of 0.6 grams of glucose solution/100 mL of distilled water
D = No addition of glucose solution/100 mL of distilled water (as a control)

RESULT AND DISCUSSION

The results of the analysis of the effectiveness of adding glucose solution to the hatching medium on embryo development and egg diameter, egg hatchability, development and growth, survival, and water quality of climbing perch larvae are presented as follows:

1. Fertilization

Fertilization is the process of uniting a female egg (ovum) with a male sperm cell (spermatozoon) to form a zygote, which becomes the beginning of embryo development. In climbing perch, this process occurs externally (outside the parent's body), namely in the water after the male and female release their gametes simultaneously during spawning.

Figure 1. Climbing perch Zygote

2. Cleavage

Cleavage is the initial stage of zygote cell division after fertilization. At this stage, the zygote divides into many small cells called blastomeres, without increasing the overall size of the embryo. The fastest cell division occurred in Treatment C at 1.20 hours after fertilization (stF), and the slowest in Treatment D at 1.26 hours stF.

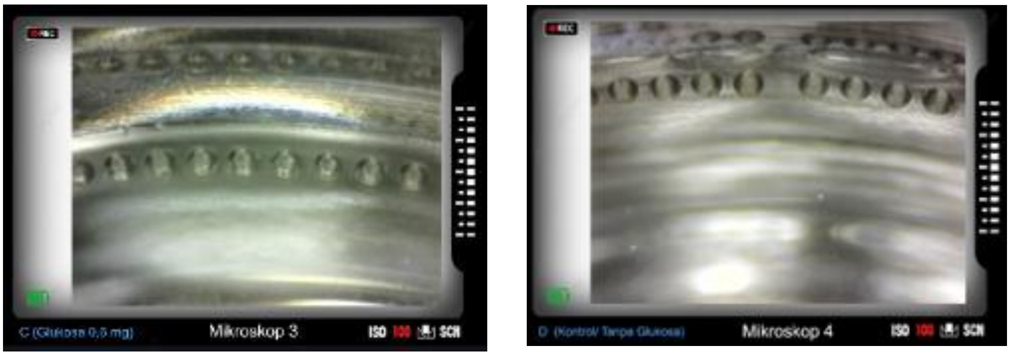

Figure 2. Cleavage of Climbing perch

3. Morula

The morula stage occurs after several cleavage cycles. At this stage, the embryo forms a dense ball composed of many small cells (blastomeres) tightly packed together. The early morula occurs at 01.44 hours stF (C) and 02.16 hours stF (D). The late morula occurs at 02.16 hours stF (C) and 02.46 hours stF (D).

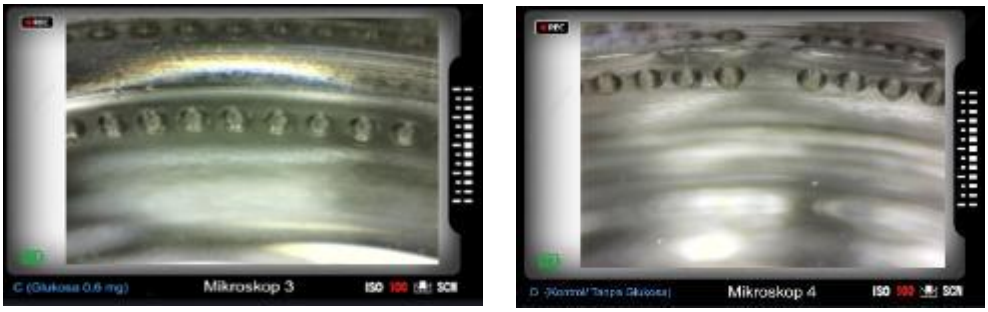

Figure 3. Morula of Climbing perch

4. Blastulation

Blastulation is the process of blastula formation, characterized by the formation of a cavity (blastocoel) within the collection of cells resulting from the division of the morula. Early blastula occurred most rapidly in treatment C, with a

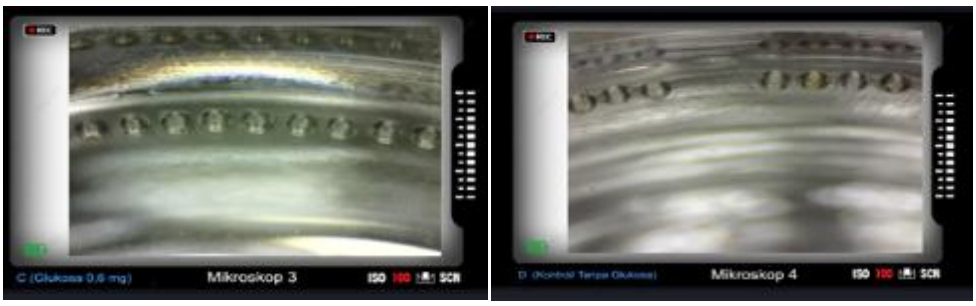

Figure 4. Blastulation of Climbing perch

latency of 03.01 hours stF, and most slowly in treatment D, with a latency of 03.50 hours stF. Late blastula occurred in treatment C with a latency of 04.07 hours and in treatment D, with a latency of 05.01 hours stF.

5. Gastrulation

Gastrulation is the process of forming three embryonic tissue layers (ectoderm, mesoderm, endoderm) through cell movement from the blastula. During gastrulation, blastula cells move inward, forming more complex embryonic structures and beginning to show body orientation (head, tail, dorsal-ventral). The initial gastrulation process requires a latency of 05.39 hours (C) and 06.01 hours stF (D).

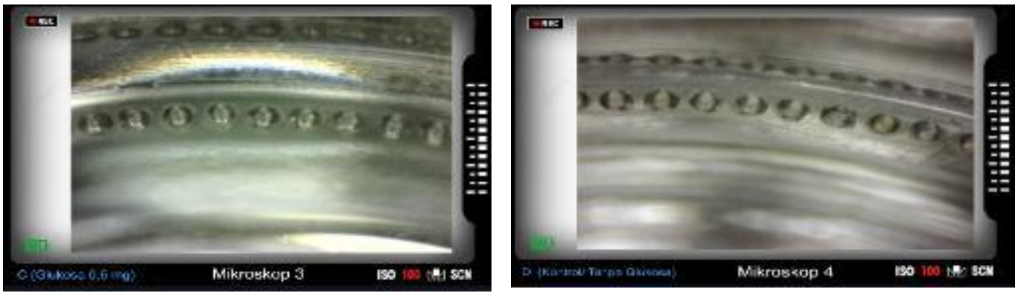

Figure 5. Gastrulation of Climbing perch

6. Organogenesis

Organogenesis is the stage of embryonic organ formation after gastrulation is complete. Cells from the three embryonic layers (ectoderm, mesoderm, endoderm) begin to differentiate to form specific tissues and organs.

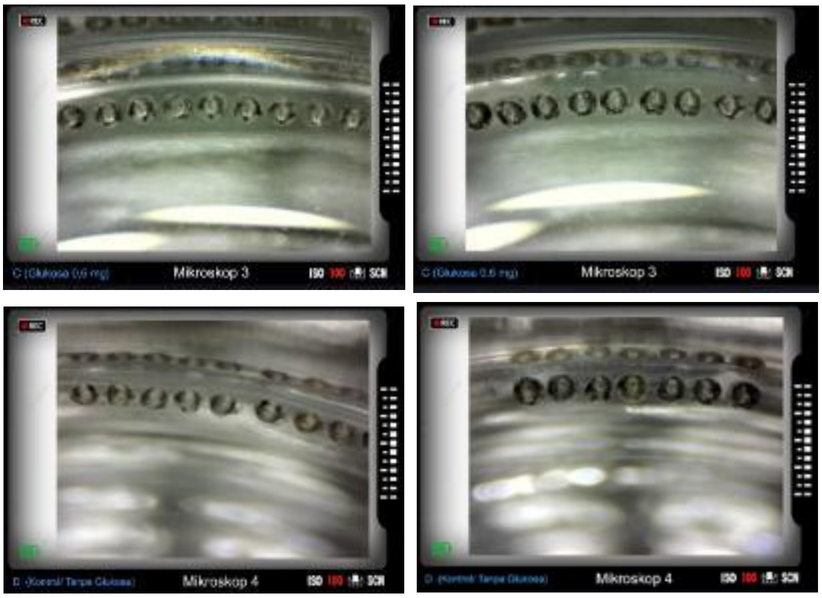

Figure 6. Organogenesis of Climbing perch

During this stage, major body structures are formed, such as:

- Head and tail
- Notochord (future backbone)
- Eyes, muscles, nervous system, and early blood circulation.

In treatment D, organogenesis occurred between 07:04 and 10:39 stF, while in treatment (D) it occurred between 07:23 and 11:01 stF.

7. Hatching

Hatching is the stage when the embryo emerges from the egg membrane (chorion) and transforms into a free-living larva. Its characteristics and process are as follows:

- Before hatching, the embryo is fully developed, with the head, tail, and major organs formed.
- The embryo secretes a hatching enzyme from a special gland in the head, which softens the egg wall.
- Once the egg wall weakens, the larva actively moves and penetrates the chorion until it is completely released.
- Newly hatched larvae still carry a yolk sac as a food reserve.

The fastest hatching process occurred at 10.50 stF in treatment C, followed by treatments A, D, and B, with times of 11.20, 11.36, and 11.44 stF, respectively.

Figure .7 Fastest hatching with a latency of 10.50 stF in treatment (C)

During the cleavage stage, the fertilized egg (zygote) begins to divide into 2, 4, 8, 16, and even dozens of cells. Treatments A and B showed slightly faster latencies (1.21–1.24 hours) than C and D (1.20–1.26 hours), although the difference was very small. This is not sufficient to demonstrate the effect of glucose, but it could be an early indication that glucose concentration may slightly contribute to the stability of the external environment.

Embryo size at the cleavage stage remained 1.0 mm across all treatments. There were no significant differences between treatments A, B, C, and D. The microenvironment within the egg remained the dominant factor. Although glucose can act as an additional energy source, the embryo is not yet able to utilize it because the egg membrane is still intact and does not allow for the absorption of external materials.

The early morula stage in treatment A appeared at 1.50 hours, B at 1.48 hours, C at 1.44 hours, and D at 2.16 hours stF. This time difference is quite interesting, as treatment C (0.6 g) appeared slightly faster than the others, while the control was the slowest. This suggests the possibility that glucose at a higher concentration provides slightly more stable osmotic conditions, thus facilitating cell division toward the morula.

Despite the time differences, the initial morula size remained at 1.0 mm in all treatments, indicating that glucose did not affect embryo size. However, the accelerated time in treatment C suggests that the glucose-treated media may increase metabolic activity around the egg, although it does not directly alter embryo size.

Treatments C and B returned to the final morula stage more quickly (2.16–2.22 hours) than those in A (2.29 hours) and D (2.46 hours) stF. A consistent pattern is emerging: glucose accelerates the morula transition process, particularly at doses of 0.4–0.6 g. The control experienced the slowest development, suggesting that the glucose-free medium may provide less than optimal environmental conditions for embryo dynamics.

In the early blastulation stage, treatment C emerged the fastest (3.01 hours), followed by B and A, while control (D) emerged the slowest at 3.50 hours. This confirms the hypothesis that glucose positively impacts media stability, allowing embryos to develop slightly more efficiently. Higher doses appear to have a more pronounced effect than lower doses.

Embryo size at the blastulation stage remained at 1.0 mm across all treatments. The difference in time without a corresponding difference in size suggests that glucose affects the rate of the process but not physical growth. Embryos continue to reorganize their internal cells without experiencing volume expansion.

At the late blastula stage, the same pattern was again observed. Treatments C (4.07 hours) and B (4.38 hours) were faster than A (4.51 hours) and D (5.01 hours). This strongly suggests that glucose accelerates blastocoel formation. The control was again the slowest, indicating that the glucose-free medium was less than optimal for cell development.

Early gastrulation in treatments A, B, and C ranged from 5.39 to 5.56 hours. Treatment D (the control) fell slightly outside this range (6.01 hours). This pattern confirms that glucose has a stimulative effect on accelerating germ layer formation. Treatment C again emerged as the fastest.

Late gastrulation showed fairly consistent differences: treatments C (6.31 hours) and B (6.54 hours) were faster than A (6.50 hours) and the control (7.08 hours) stF. High glucose doses (0.6 g) sequentially had the greatest acceleration effect, followed by B and A. The control treatment was again the slowest.

Initial organogenesis (neurulation) in treatments C and A occurred almost simultaneously (7:04–7:06 hours), followed by B (7:11 hours), while the control remained the slowest (7:23 hours). The consistency of the control lag at each stage indicates that the presence of glucose does contribute to the efficiency of embryonic development.

Embryo size remained 1.0 mm in each treatment. This indicates that glucose only affects developmental speed, not embryo size. Physical growth occurs primarily after larvae hatch, when they begin to utilize energy from the environment.

The somite formation stage in treatments B and C (8:34–8:55 hours) was again faster than A (0.47 hours noted as a possible typographical error) and the control. At this stage, all embryos had entered the stage of muscle and skeletal structure formation. The acceleration in treatment C indicates a better metabolic rate in the high-glucose medium.

The formation of major organs (eyes, heart, gills, and initial pigmentation) showed a similar pattern: treatments C and B occurred within 9.46–10.15 hours, faster than A (9.51 hours) and the control (10.06 hours). The control showed a small but consistent delay, indicating that the glucose-free medium provides a less efficient developmental environment.

Initial pigmentation appeared earlier in treatment C, suggesting that high glucose concentrations may provide a metabolic stimulus around the egg surface. Embryo size remained similar across all treatments, confirming that glucose does not affect structural growth during the embryonic stage. Yolk closure occurred most rapidly in treatment C (10.39 hours), followed by A and B (10.53–10.55 hours), while the control was the slowest (11.01 hours). This suggests that glucose has the potential to accelerate yolk utilization, although yolk remains the primary energy source.

Treatment C consistently emerged the fastest at almost all stages, indicating that the 0.6 g glucose dose provided the most optimal environmental conditions for embryo development. Treatment B came in second, followed by A, while the control consistently lagged behind. This confirms that the presence of glucose in the media accelerates embryo development, although it does not affect size.

The addition of glucose affected embryo development speed, but not embryo size. A higher glucose dose (0.6 g or treatment C) was most effective in accelerating each developmental phase, while the control treatment (without glucose) consistently lagged behind in developmental time. The results indicate that glucose can increase the efficiency of the external environment, although the vitellus remains the primary energy source for climbing perch embryos until hatching.

Andini et al. (2022) in tawes fish, where energy supplementation through external media (honey in NaCl) significantly increased hatchability and larval survival. Additional energy in the hatching media can increase the metabolic efficiency of embryos and support more stable development. Research on the climbing perch reinforces this concept: glucose as a media additive can improve the quality of embryonic development, particularly by accelerating the morula, blastula, gastrulation, and organogenesis stages. Glucose has the potential to be an alternative additive in freshwater fish hatching media to increase embryonic development efficiency without affecting embryo size. Glucose is a direct energy source for embryos, accelerating cell differentiation (Berling et al., 2024).

Rukmini et al. (2014) found that climbing perch embryonic development is strongly influenced by water quality and energy availability. The water quality in this study, which was within the optimal range (temperature 27–28°C, pH 6.2–7.0, DO 2.9–5.1 mg/L), supported consistent hatching success. Mandal et al. (2016) demonstrated that climbing perch spawning and hatching success can reach >95% if hormonal, environmental, and physiological conditions are favorable. Climbing perch embryos have high hatchability potential, and glucose supplementation at certain concentrations can act as a supporting factor but is not the main factor determining the success of climbing perch egg hatchability. Hasan (2017) added simple sugars such as honey or glucose can increase hatchability, survival, and early growth of various fish larval species.

CONCLUSION

The addition of glucose solution to the hatching medium has a positive effect on embryo development. Glucose helps increase the metabolic activity of the embryo, accelerates development, and produces optimal hatching power (100%).

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