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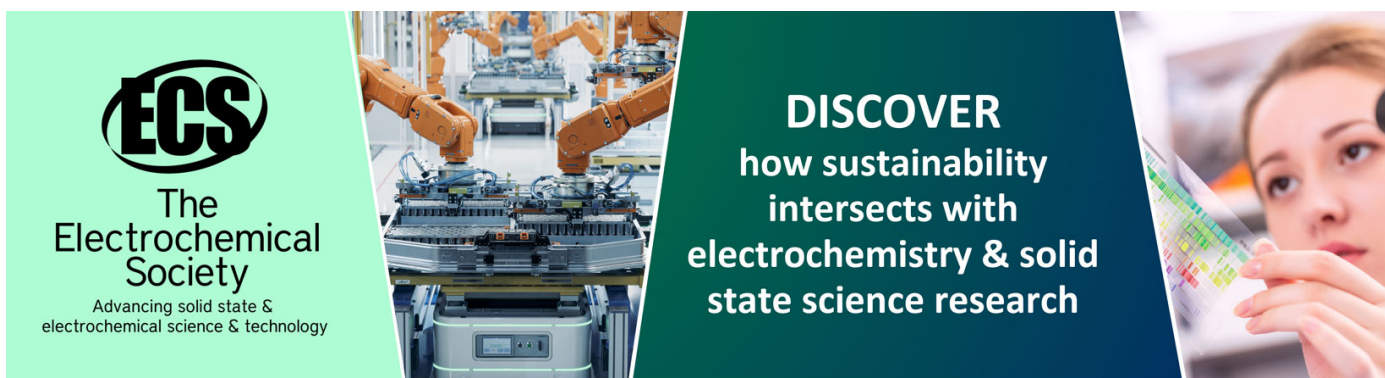
The use of bromelain enzyme on artificial hatching media as an effort to hatch Nile tilapia (*Oreochromis niloticus*) eggs outside the mother's mouth

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The use of bromelain enzyme on artificial hatching media as an effort to hatch Nile tilapia (*Oreochromis niloticus*) eggs outside the mother's mouth

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Abstract. Nile tilapia is a type of mouthbreeder fish which is a fish that incubates its eggs in the mouth. The parent fish that do spawning must incubate eggs for up to 12 days and fast until the eggs in their mouths hatch, it can cause nutrient deficient and a long recovery period that will affect the subsequent production. The conditions on artificial tilapia eggs hatching media are made to resemble the conditions in the mouth of tilapia, one of which is the presence of the protease enzyme. One of the protease enzymes that can be used is the bromelain enzyme found in pineapple. This study used the experimental method and used a completely randomized design (CRD) trial with three replications and five treatments. Bromelain enzymes used were A (0, 09 μmol), B (0.105 μmol), C (0, 12 μmol), D (0.135 μmol) and E (0.15 μmol). The parameters observed were hatchability and survival rate. The results suggested that the bromelain enzyme can be used to do tilapia egg hatching outside the mother's mouth with the highest hatching rate was 74.44%. But it does not affect the survival rate of tilapia larvae with a survival rate was 82.5%.

1. Introduction

Nile tilapia (*Oreochromis niloticus*) is one of the most widely cultivated freshwater fish in Indonesia. Tilapia ranks second after carp (*Cyprinus carpio*). Tilapia is also an important fish in aquaculture. Tilapia is an important fish in the world because tilapia consumers are found on various continents. Red tilapia is even used as a substitution (substitute) for red snapper (Sea bream) because of its similar appearance; therefore, tilapia is referred to as freshwater snapper [1].

Factors that play an important role in the prospect of tilapia are the distinctive taste of the meat, the clean white color of the meat and the high nutritional content, so that it is often used as a source of protein that is cheap and easy to obtain, and has an affordable selling price by the community [2]. Tilapia is a mouth breeder fish where these fish incubate and care for fish larvae in the mother's mouth. Females incubate eggs in the mouth to protect from predators so that the eggs can hatch properly. Tilapia incubates their eggs for up to 12 days at the age of 6-7 days after hatching larva begins to be released by the mother. In this process, tilapia will fast until the egg in the mouth hatches and can find its own food. Fasting mothers will experience weight loss and are susceptible to disease [3].

Based on the test results of the protease enzyme content in the mouth of tilapia that are incubating eggs found that in the saliva of tilapia there is a protease enzyme content of 0.09 μmol . The



temperature in the water that is in the mouth of tilapia is the same as the ambient temperature because the fish has poikilothermic properties, that is, the body temperature adjusts to the ambient temperature. Protease enzyme is enzymes that function to break down proteins by hydrolyzing peptide bonds to amino acids in the polypeptide chain. According to [4], the bromelain enzyme is one group of protease enzymes where the bromelain enzyme has the ability to hydrolyze peptide bonds to proteins or polypeptides into smaller molecules, amino acids. Bromelain enzyme is obtained from pineapple extract. This study aims to determine the effect of different doses of bromelain enzymes in hatching tilapia on the hatchability of tilapia eggs. In order to get the best dose of bromelain enzyme used in hatching tilapia eggs.

2. Materials and methods

2.1. Experiment method and design

This research method uses an experimental method using a completely randomized trial design (CRD) consisting of 5 treatments namely A (0.09 micromoles), B (0.105 micromoles), C (0.12 micromoles), D (0.135 micromoles) and E (0.15 micromoles). The study was conducted using incubation hatches made from 320 ml bottles.

2.2. Research procedures

2.2.1. Making hatching incubation

Nile tilapia eggs were hatched using a hatching incubator. The incubator for hatching tilapia eggs is made from light bulb bottles with a bottle volume of 320 ml. At the top of the bottle two holes are given in the first hole given aeration hose which aims to enter the air from the aerator and the second hole aims to remove the air entering the bottle so that the air pressure in the bottle is stable. At each hose in the middle is given a tool to adjust the volume of air released. At the end of the aeration hose is connected to the aeration pump with a power of 4 liters / minute.

2.2.2. Container preparation

Preparation of the study began with preparing tools used such as pumped water, hoses and 320 ml bottles. The instrument used is cleaned by using detergent and then dried for 24 hours. Clean tools and containers are placed in the hatching rack in accordance with the plan of the randomized trial that has been made.

2.2.3. Media preparation

The media used in this study is clean fresh water that has been deposited for 24 hours. After being deposited water is given a treatment in the form of a commercial bromelain enzyme which aims to speed up the time of hatching of eggs. Doses used in this study were 0.09; 0.105; 0.12; 0.15 micromoles. Water media containing enzymes in accordance with each treatment was put into 300 ml bulb bottles. Bromelain enzymes were given in the media by using solution dilution. According to [5], the dilution formula is as follows:

$$V_1 \times C_1 = V_2 \times C_2$$

Information:

V1: Solution Volume 1

V2: Solution Volume 2

C1: Solution concentration 1

C2: Solution concentration 2

2.2.4. Test sample preparation

The sample used in this study was tilapia eggs. Tilapia eggs are obtained from the mouths of tilapia that have spawned. Retrieval of fish eggs using the knock method or egg collection by opening the mouth of the tilapia.

2.2.5. Maintenance of larvae

Nile tilapia larvae that have been laid out from their eggs are transferred into different containers. The tilapia maintenance container is given aeration to supply oxygen to the water media. During the maintenance of tilapia larvae are not given food because tilapia larvae still have yolk sacs as nutrients that are absorbed by the body.

2.3. Test parameters

2.3.1. Main parameters

A. Egg hatchability

The main parameter is the success of hatching eggs in tilapia (*Oreochromis niloticus*). Egg hatchability is the percentage of eggs that mature after the eggs are fertilized. According to [6], hatching rate is the percentage of hatching eggs that can be known and calculated using the formula, namely:

$$\text{HR (\%)} = \frac{\text{hatched egg}}{\text{Total egg}} \times 100\%$$

B. Survival rate

Another supporting parameter observed was survival rate. Observation of survival rate parameters is done after the maintenance of tilapia larvae for seven days or when the yolk of the tilapia egg yolk has run out. This observation was made by comparing the presentation of the number of fish that survived during maintenance with the number of fish that hatched. According to [7], the fish survival rate formula is as follows:

$$\text{SR (\%)} = \frac{\text{Larvae (t)}}{\text{Larvae (0)}} \times 100\%$$

2.4. Data analysis

Analysis of diversity or F test is done if the F value is significantly different or very real, then to compare the values between treatments followed by the BTN test to determine the treatment that gives the best effect. The best influence on the level of confidence of 1% to determine the relationship between treatments used orthogonal polynomial regression analysis.

3. Results and discussion

3.1. Hatchability

The hatching of eggs is the last phase of the development of egg embryogenesis. The egg is said to hatch when an embryo that has developed perfectly and comes out of the eggshell. This is due to mechanical movements and enzymatic reactions that occur in the egg. This study got a presentation of hatchability of tilapia eggs in each treatment can be seen in Figure 1.

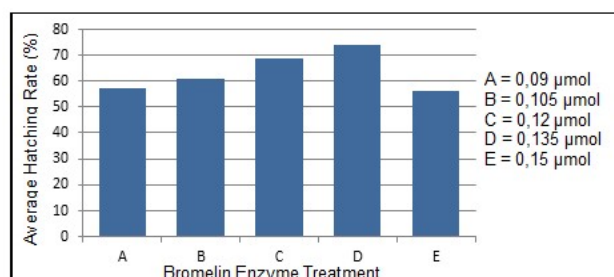


Figure 1. Average of egg hatchability.

The data can be concluded that the treatment that produced the best hatchability was in treatment D with an average value of 74.44% hatchability. The next treatment that has an average hatchability value of 68.89% is treatment C. The next best treatment is treatment B with an average value of 61%. The lowest hatchability value 2 is treatment A and E.

Treatment A has an average hatchability value of 57%, this is because treatment A has the lowest dose compared to other treatments, so the process of eroding the *chorion* portion of the egg becomes lower compared to other treatments. The E treatment had the highest dose of the bromelain enzyme. According to [8], the hatchability of fish eggs given a dose of pineapple extract containing the bromelain enzyme in accordance with the dose will give a high hatchability value. The higher dose of bromelain enzyme in the hatching medium will reduce the hatchability of the egg. The amount of bromelain enzyme content that is too high will damage the development of the embryo.

Data analysis using variance showed that the calculated F value was greater than the F5% and F1% values. The data obtained was then performed LSD test and orthogonal polynomial test. Orthogonal polynomial testing is used to determine the effect of the relationship between treatments. The results of the regression curve are in Figure 2.

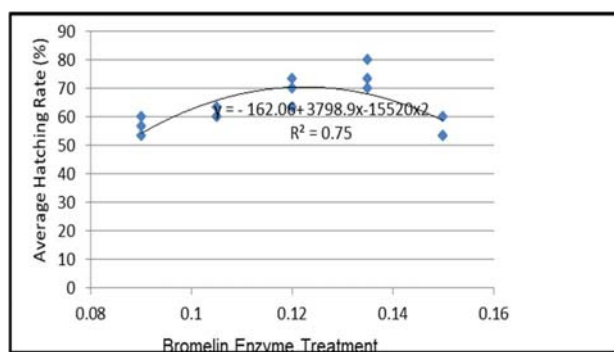


Figure 2. Hatching regression curve.

The graph above can be concluded that the results of the graph obtained with quadratic curve pattern with the value of the equation $y = -162.06 + 3798.9x - 15520x^2$ with the value $R^2 = 0.75$. $R^2 = 0.75$ indicates that the hatchability of tilapia eggs in each treatment is 75% influenced by enzymes while the other 25% is influenced by other factors such as temperature, DO and egg quality. The relationship can be concluded that the best treatment is in treatment D with a bromelain enzyme dose of 0.135 µmol. The dose value at the peak point obtained from the curve by differentiating the equation obtained the dose value of 0.1223 µmol. The higher amount of enzyme will cause damage to the egg structure due to erosion by the enzyme. According to [9], the use of proteolysis enzymes can significantly increase hatching and shorten the hatching time. However, the amount of enzyme content that is too high will damage the outside and reduce the hatchability of eggs. This is in accordance with

statement [10], damage to the outer layer of the egg (*chorion*) can be caused by the high content of the enzyme bromelain contained in the treatment solution.

3.2. Survival rate

The treatment of giving bromelain enzymes with different doses on the performance media of tilapia eggs carried out maintenance period of tilapia larvae for 7 days. Tilapia larvae in the first 7 days of maintenance can take nutrients from egg yolk so that no artificial feeding of tilapia larvae is carried out. Data on average survival rate can be seen in Figure 5.

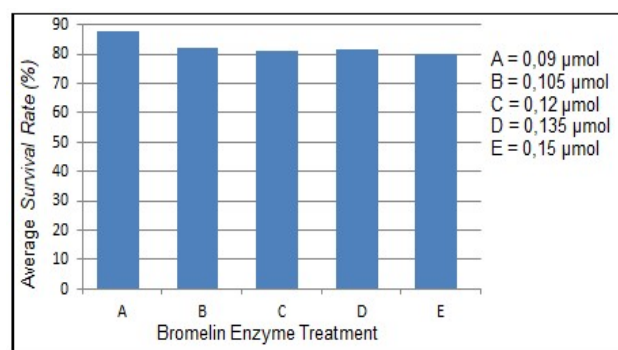


Figure 3. Data on the survival rate of tilapia larvae.

Based on the above data it can be seen that the lowest average survival rate is in treatment E with an average survival rate of 80.05%, then followed by treatment C with an average value of 81.04%, after that treatment D and B with an average value of 81.6% and 81.9%. The treatment that has the highest survival rate is treatment A with an average survival rate of 87.9%. Different dosages of Bromelain enzyme do not affect the survival rate of tilapia. This is evidenced by the results of various survival rate data shows that the F count is smaller than the F5% and F1%. This is in accordance with statement [10] which stated that the treatment of pineapple solution does not affect the survival rate of larvae. Several factors that affect the survival rate of an organism include competition between species, lack of food, parasites and the ability to adapt to the environment. The survival rate of fish is high if the quality and quantity of feed and the environment are supportive.

4. Conclusions

Based on the results of this study it was concluded that the administration of bromelain enzymes with different doses in the media of hatching tilapia (*O. niloticus*). The best results of egg hatchability obtained by treatment D with a dose of the enzyme bromelain 0.135 µmol. The average value of hatching power obtained is 74% with the value of the equation $Y = -162.06 + 3798.9x - 15520x^2$ and the value of $R^2 = 0.75$. From the equation, the maximum X value is 0.1223 µmol. The dose of bromelain enzyme does not affect survival. Suggestion given from the results of this study is to do artificial hatching of tilapia eggs using the enzyme bromelain is recommended to use a dose of 0.1223 µmol. Further research needs to be done on the application of the enzyme bromelain to the hatchability of other mouth breeder fish eggs. So the use of bromelain enzymes is more widespread in the field of aquaculture.

5. References

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