Gonado Somatic Index inG5Transgenic Mutiara Catfish *(Clariasgariepinus)*At Different Temperatures

ABSTRACT

Temperature is one of the factors that can affect the maturity of the gonads in fish. This study aims to find out what is the best temperature for maturation of broodstock to prepare G5 transgenic mutiara catfish broodstock that are ready to hatch based on gonado somatic index (GSI) performance, relative fecundity, fertilization rate (FR), hatching rate (HR). The research was carried out in the Hatchery Building 4, Fisheries Biotechnology Laboratory, Faculty of Fisheries and Marine Sciences. The method used in this research is an experimental method with a complete randomized design (CRD), which consists of four treatments and three replicates. Treatment A: 26 ± 0.5 °C, B : 28 ± 0.5 °C, C : 30 ± 0.5 °C for transgenic catfish, C*: 30 ± 0.5 °C (for nontransgenic catfish) with maintenance for 8 weeks. The reproductive performance of the female broodstock of G5 transgenic mutiara catfish with the highest relative fecundity was found in treatment B of kg/brood. The best temperature for gonad maturation was obtained in treatment B with a temperature of 28± 0.5 °C with *a gonadosomatic index*value *of* 52.42 ± 5.93% for females and 5.22 ± 0.17% for males. The spawning performance in the parent pair of treatment B produced the best relative fecundity, fertilization rate (FR), and hatching rate (HR), with values of 89,179 eggs/kg, 84.98 ± 5.01%, and 90.87 ± 3.12%, respectively.

Keywords: gonad maturation, transgenic catfish, temperature manipulation, G5

1. INTRODUCTION

Catfish culture activities are one of the cultivation in the fisheries sector that continues to experience rapid development. This is because catfish is one of the fish that is in great demand by the community. Natural spawning is the spawning that is most carried out by culture. However, this natural spawning will produce a smaller quantity of fish fry than artificial and semi-artificial spawning, besides that natural spawning also tends to experience many failures. Spawning by utilizing hormones and natural compounds has been widely carried out and has shown significant results [1]. The hormone ovaprim, which is often used for catfish spawning, has also been shown to significantly increase ovulation [2]. Problems that occur in natural spawning occur because natural spawning depends a lot on the readiness of mature broodstock and generally only occurs in certain seasons.

Catfish spawning using hormones can be done to make the spawning process easier. The use of hormones can make it easier for farmers to be able to produce fingerlings outside of the spawning season, where the fish to be spawned have gone through the previous maturation process until they are ready to spawn [3]. The maturation process in the broodstock that is not optimized will make it difficult for the fish to mature the gonads at a certain time. One of the efforts to optimize the broodstock can be done by environmental manipulation.

One of dominant factors that influence fish gonadal maturity is temperature. In addition, temperature can also affect the reproductive activity of fish because it can increase or decrease the weight of the gonads. Previous research to relatedenvironmental factors that have a significant role in influencing the development of fish gonads are temperature, food, light, and season[4]. In the maturation process, the ideal temperature to maintain gonad maturation in catfish groups is 27-30°C [5]. Maturation must be accompanied by spawning induction, because maturation can be considered successful if the broodstock that is matured with temperature can be induced to spawn. The indicator can determine how much temperature affects the fecundity that will produce the number of eggs and larvae to be produced.

The increase in egg production is shown by fecundity. The quality of fecundity depends on a good broodstock strain. Transgenic mutiara catfish*(Clariasgariepinus*) strains produced from the use of transgenic technology (addition of exogenous GH, *Clariasgariepinus Growth Hormone, CgGH*) have higher reproductive performance than non-transgenic catfish. The propagation of transgenic strain catfish through the breeding process has produced the transgenic mutiara catfish G5 [6, 30]. Research showed by the transgenicG1 and G2 mutiara catfish could produce more catfish fingerlings than non-transgeniccatfish[7]. Currently, transgenic mutiara catfish have reached the fifth generation (G5). Therefore, it is necessary to carry out research on transgenic and non-transgenic fish with different temperature variations.

2. MATERIAL and METHODS

The research was carried out in the Hatchery Building 4, Fisheries Biotechnology Laboratory, Faculty of Fisheries and Marine Sciences, Padjadjaran University. The time for conducting the research is from May to August 2023 which starts from preparation, implementation, and observation.

The tools used for the research are, round fiber tubs, heaters, thermometers, aeration installations, water pumps, brushes, basins, scales, bars, sterofoam, injections, kakaban, black plastic, containers, cameras, plastic containers, label paper. The ingredients used are test animals, Prima Feed (PF)128 broodfish feed, ovaprim hormone, aquabides, chorulon hormone (hCG, *human chorionic gonadotropin*).

The method used in the study was an experimental method with a Complete Random Design (CRD) consisting of four treatments and three replicates. The temperature treatments used were as follows: A (26 ±0.5 °C), B (28 ±0.5 °C), C (30 ±0.5 °C) for G5 transgenic mutiara catfish and C* (30 ±0.5 °C) for non-transgenic catfish as a controlto compare with fish that do not have growth hormon.The parameters used during the research were as follows:

a. Gonado Somatic Index (GSI)

The success of the gonad maturation process can be presentedfrom the Gonado Somatic Index (GSI) value which reflects the growth of female or male gonads. The gonadal maturation rate calculated using the GSI value reflects whether the parent can be castrated or not. The calculation of the GSI value is calculated using the following formula according to [8,31]:

$$GSI = \frac{Gw}{Bw} \times 100\%$$

Information:
GSI = Gonado Somatic Index (%)
Gw = Gonadal weight (g) (weight before spawning – weight before after spawning)
Bw = Body weight (g)

b. Relative Fecundity (RF)

Fish fecundity can be calculated in several ways, namely: the number method, the volumetric method and the gravimetric method. This study uses the gravimetric method [9] in the following way:

$$RF = \frac{Wg}{We} \times 100\%$$

Information:
RF = Relative fecundity
Wg = weight gonad (g)
We = Mean weight of egg sampel (g)

c. Fertilization Rate (FR)

The rate of fertilization is calculated as the percentage of the number of eggs fertilized (undergoing the embryogenesis process) compared to the number of eggs from spawning calculated on a sieve as a container for treatment samples. Fertilized catfish eggs are yellow-green, while unfertilized eggs are milky white or pale. Fertilized eggs are counted after the fertilization process. The rate of fertilization is calculated using the formula Effendie (1997), as follows:

$$FR = \frac{\sum \text{ fertilized eggs}}{\sum \text{total } egg} \times 100\%$$

d. Hatching Rate (HR)

The rate of hatching is calculated as a percentage of the number of eggs hatched compared to the number of fertilized eggs. The calculation of hatching rate is carried out 18 - 24 hours after the fertilization process (at the water temperature of the hatching medium ranges from 29°C-30°C). The rate of hatching is calculated using the formula [10], as follows:

$$HR = \frac{\sum \text{ fertilized eggs}}{\sum \text{ total egg}} \times 100\%$$

e. Data Analysis

The data from the research results were analyzed quantitatively using one way ANOVA with advanced tests Duncan's Multiple Range Test (Sigma plot 14.5)

3. RESULT AND DISCUSSION

3.1 Female Gonado Somatic Index

Observation of the Gonado Somatic Index (GSI) for eight weeks in female G5 mutiara and non-transgenic catfish in the form of percentages (%) is presented in (Figure 1).

The results of gonad maturation with temperature treatment showed that the highest female gonado somatic index value was found in treatment B (temperature 28 ± 0.5 °C) with a gonado somatic index value of 52.42 ± 5.93% followed by treatment A (temperature 26 ± 0.5 °C) with a gonado somatic index value of 43.54 ± 8.55%, treatment C (temperature 30 ± 0.5 °C) with a GSI value of 42.39 ± 10.60%, while the lowest GSI value of the female is found in the treatment C* (temperature 26 ± 0.5 °C) by producing a gonado somatic index (GSI) value of 19.02 ± 2.28%.

The growth of the gonads of transgenic mutiara catfish can be optimized by using temperature. In Lin's research, the temperature of 28°C has the best influence on gonadal growth[11]. The high GSI value in treatment B females is one of the reasons for the use of the optimum maintenance temperature of 28°C. In addition, the rapid growth of fish can affect gonadal growth. Transgenic mutiara catfish in treatment B had the highest GSI value also influenced by the presence of exogenous GH inserts. Transgenesis technology has been carried out [12] to produce G0 transgenic mutiara catfish through transgenesis technology by increasing growth by two and a half times (125%) compared to non-transgeniccatfish. Exogenous GH inserts have a positive effect on the reproduction of G1 transgenic mutiara catfish, namely it can increase gonadal fertility, increase egg size and increase the production of estradiol hormone [13].

Temperature fluctuations are thought to have an effect on the *CgGH* excretion level. The temperature of 28°C produced the highest GSI value because at 28°C it was related to a high level of expression which when compared to treatment A (26°C) and treatment C (30°C). And vice versa, the low GSI value obtained at temperature 26 is suspected to be due to a decrease in the expression level of *CgGH*. Rapid growth can affect the value of GSI. The

effect of *CgGH* on fish gonad growth related to the induction of exogenous GH stimulates the secretion of IGF-1 growth factor which works in cooperation with GH and steroids (estrogen, E2 or testosterone, T) to multiply gamete cells (sperm or ovum). The multiplication of these gamete cells leads to an increase in gonadal growth as a consequence of which the GSI value increases at the optimum temperature of 28°C. Previous research on the G1 transgenic mutiara catfish, showed that the growth of the gonads of transgenic fish was higher when the broodstock was kept in the temperature range of 27-28°C [14].

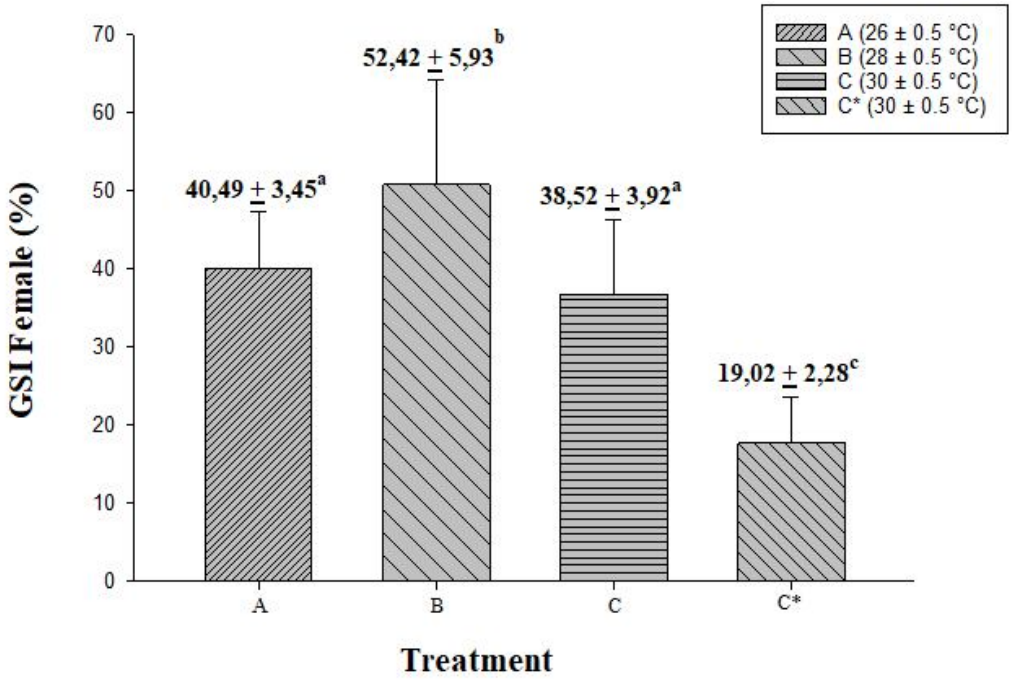

Fig 1. Graph of GSI values of transgenic mutiara catfish G5 (A,B,C) and non-transgenic (C*). The mean followed by different letter notations showed significance (p<0.05)

3.2 Male Gonado Somatic Index

The observation results during 8 weeks of maintenance can be seen in (Figure 2)

The highest male gonado somatic index value was found in treatment B (temperature 28±0.5°C) with a gonadosomatic index value of 5.22 ± 0.17%, followed by treatment C (temperature 30±0.5°C) with a gonado somatic index value of 5.13 ± 0.36, and treatment A (temperature 26±0.5°C) of 4.92±0.37%. Meanwhile, the C* treatment (temperature 30±0.5) obtained the lowest GSI value with a male parent GSI value of 1.95 ± 0.96%. The difference in treatment A, B, C with treatment C* is suspected because in transgenic mutiara catfish there is an insert of the GH gene [14]. Exogenous GH has a positive effect on the reproduction of G1 transgenic mutiara catfish, namely it can increase gonadal fertility, increase egg size and increase the production of estradiol hormone [13].The results of the research that have been carried out show that the gonado somatic index value in the male broodstock of transgenic mutiara catfish is better compared to the gonado somatic index value in non-transgeniccatfish. The gonado somatic value of the male brood index in non-transgeniccatfish obtained is still relatively good because it has a value of 1.95 ± 0.96%. The gonado somatic value of the male broodstock index of mutiara catfish ranged from 0.22%-1.47% [15]. Meanwhile, the GSI

value of male catfish in the waters of the Nile is reported to be around 1% [16]. The GSI value of male broodstock in the G4 transgenicmutiara catfish research got the best score of 3.55 ± 0.24% [17]. In this study, the G5 transgenic mutiara catfish had a gonado somatic index value of 5.22 ± 0.17%. This value is much higher compared to non-transgenic catfish. A significant difference between transgenic mutiara catfish and non-transgenic mutiara catfish is suspected because transgenic mutiara catfish have GH gene inserts [15]. Exogenous GH has a positive effect on the reproduction of G1 transgenic mutiara catfish, namely it can increase gonad fertility [13]. GH in male fish spurs the secretion of testosterone produced by leydig cells which functions to carry out the process of spermatogenesis until it becomes spermatozoa or ripe sperm [18].

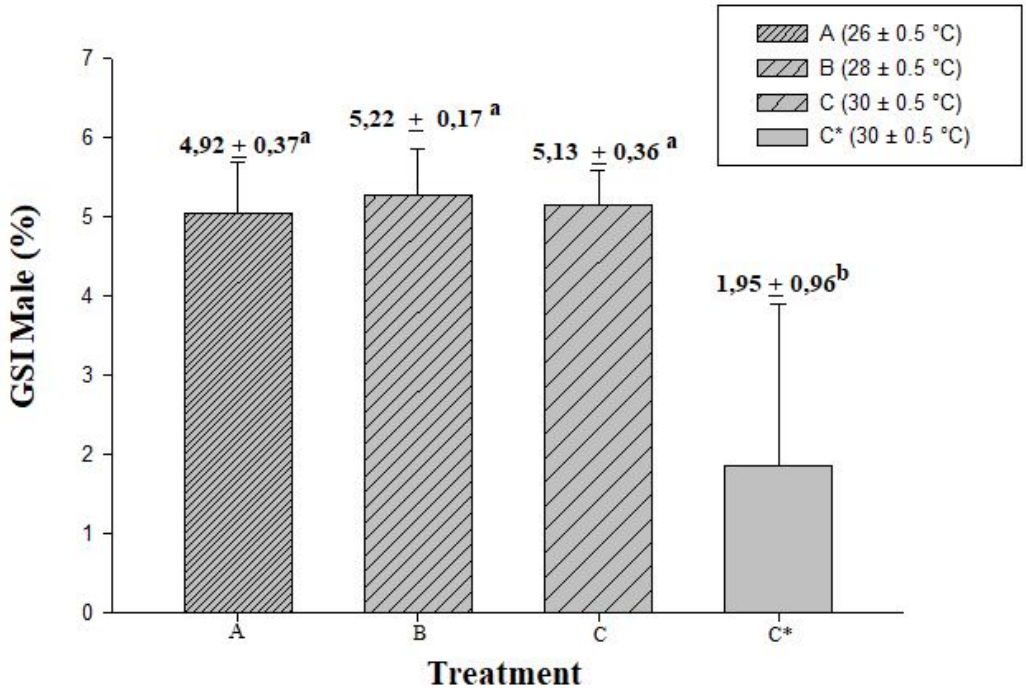

Fig2. GSI value chart of male transgenic mutiara catfish G5 (A,B,C) and non-transgenic (C*). The mean followed by different letter notations showed significance (p<0.05)

3.3 Relative Fecundity

The relative fecundity obtained from fish that successfully spawned during the 8 weeks of the study can be seen in Table 1.

Based on Table 1, the relative fecundity of the broodstock catfish of the G5 transgenic mutiarabenita was highest in treatment B (♂ B1 X ♀ B3) of 89,179 egg/kg of broodstock, followed by treatment A (♂ A3 X ♀ A2) of 87,530 and treatment C (♂ C2 X ♀ C2) of 83,857. According to Putri research, transgenic mutiara catfish in the first offspring produced a relative fecundity of 83,333-84,000 egg/kg[13].

In the study, G4 transgenic mutiara catfish succeeded in spawning with a relative fecundity of 85,103 eggs/kg [17]. The results of the research on the spawning of G5 transgenic mutiara catfish, obtained the highest value of 89,179 egg /kg. This value is classified as a good number of fecundity [19] which states a good fecundity value for catfish, which is around 50,000-100,000 egg/kg of fish body weight.

The success of G5 transgenic mutiara catfish in spawning in a relatively faster time with a good fecundity value is because in the transgenic mutiara catfish there is a GH insert of *C. gariepinus* [12]. One of the factors that

can affect the fecundity of fish is the age of the fish. The age of fish affects the development of the gonads, in male fish maturing the gonads generally experience gonadal maturation at an earlier time, namely 8 months [3]. Meanwhile, the gonads of female fish mature at the age of 10 months and the spawning cycle is once every 1.5 months [20,32]. Meanwhile, in female fish, the physical characteristics are the enlargement of the abdomen and when striping is carried out, there are eggs that come out.

Table 1. Relative Fecundity

Treatment	Relative Fecundity (egg/kg broodstock)
(A) ♂ A3 X ♀ A2	87,530
(B) ♂B1 X ♀ B3	89,179
(C) ♂ C2 X ♀C2	83,857

3.4 Fertilization Rate

The results of the fertilization rate in catfish broodstock that successfully spawned after eight weeks of rearing can be seen in Figure 3.

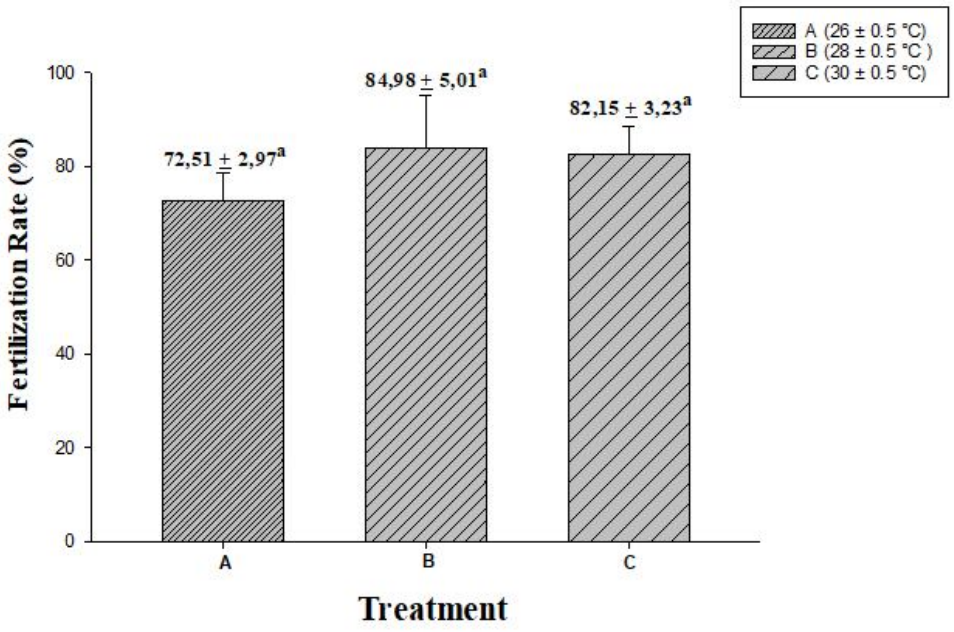

Fig 3. Graph of fertilization rate of G5 transgenic mutiara catfish eggs (A,B,C). The average followed by different letter notation shows significance ($p<0.05$)

Based on Figure 3, the highest fertility value in G5 transgenic mutiara catfish was in treatment B, which was 84.98 ± 5.01, followed by treatment C of 82.15 ± 3.23 and the lowest value was in treatment A of 72.51 ±. 2.97. The value of the rate of fertilization is still relatively good, referring to the Indonesian National Standardization (SNI) [19] no. 01-6484.3.2000, catfish have a fertilization rate in the range of 70% to 90% [21]. The rate of fertilization that reaches a value above 70% is categorized as high [22]. Data analysis resulted in Figure 4, the rate of fertilization between cross C and cross A and B does not have a significant difference, This is suspected because the gonad maturity level of the cross pair of G5 transgenic catfish broodstock is relatively uniform. Allegedly, this is the cause of the rate of hatching in each egg produced in the G5 transgeniccatfish parent pair is relatively the same. Furthermore, in non-transgenic mutiara catfish that use temperature treatment, it does not cause maturation in the broodstock so that it causes spawning failure.

3.5 Hatching Rate

The following are the results of the rate of egg hatching that can be seen on (Figure 4).Based on Figure 4, the data on the rate of hatching of G5 transgenic mutiara catfish eggs shows that the highest value of hatching rate is in treatment B of 90.87±3.12 followed by treatment C of 87.92±5.23 and the lowest value is in treatment A of 80.97±4.93. Hatching rate value Transgenic mutiara catfish is relatively high compared to sangkuriang catfish used in artificial spawning in Poland with a value of 66.14%-68.50% [23] and in Nigeria which is 84.15 ± 2.05% [24] and around 60%-66% [25].

The rate of hatching in each treatment can be categorized as high according to the research of Hijriyanti [26] which states that the rate of hatching in the range of 30% - 50% is considered low, and > 60% is considered high. The high value of the rate of egg hatching is due to the maximum production of eggs. Stated that the success of hatching is influenced by the quality of the eggs and the number of fertilized eggs, if the maximum yield will also be maximum [27]. In addition, according to Marnis [28], the transgenic *Pangasianodonhypophthalmus* growth hormone(*PhGH*)present in transgenic fish is also influential in producing a high rate of hatchability, which is 80.68%, while non-transgenicfish is only 76.68%[29]. This is directly proportional to thisstudy, where the G5 transgenicmutiara catfish obtained a result of above 80% with the best score of 90.87%.

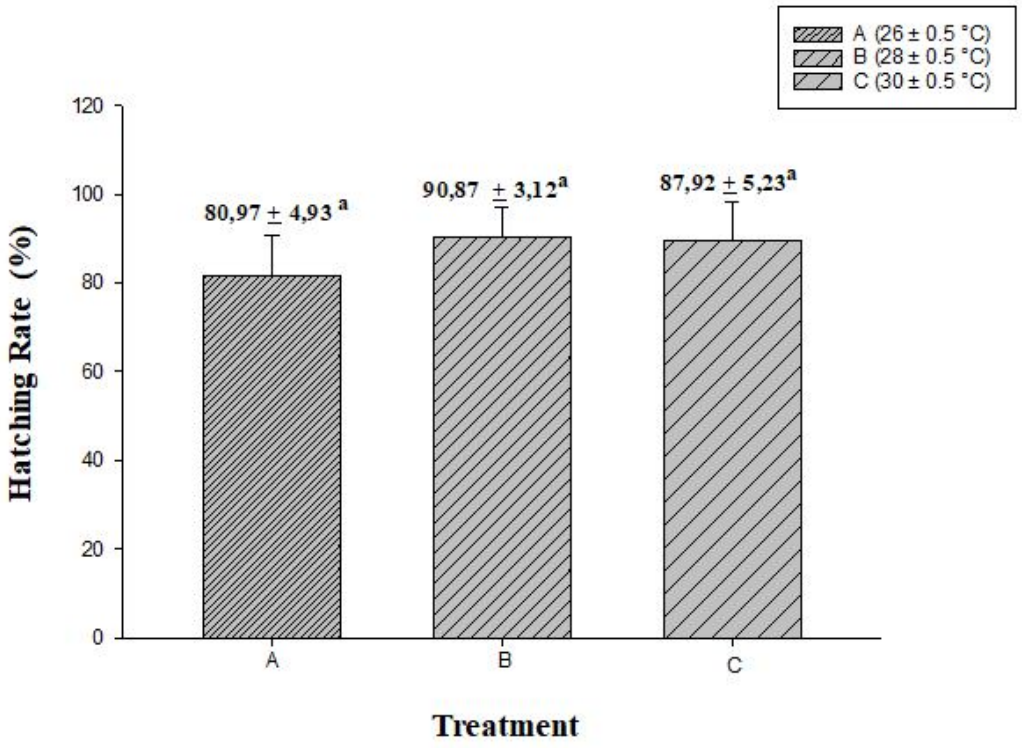

Fig 4. Graph of hatching rate of G5 transgenic mutiara catfish eggs (A,B,C). The average followed by different letter notation shows significance (p<0.05)

4. CONCLUSION

The best temperature for gonad maturation was obtained in treatment B with a temperature of 28 ± 0.5 °C with a gonadosomatic index value of 52.42 ± 5.93% for females broodstock and 5.22 ± 0.17% for male broodstock. The broodstock pair of treatment B also produced the best relative fecundity, fertilization rate (FR), and hatching rate (HR) values with sequential values of 89,179 egg/kg broodstock, 84.98 ± 5.01%, and 90.87±3.12%.

In the next study, it was recommended to use a temperature of 28 ± 0.5 ° C to accelerate the maturation of the gonads in catfish broodstock.

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