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IMPROVE SPAWNING QUALITY OF KISSING GOURAMI (*HELOSTOMA TEMMINCKII*) WITH COMMERCIAL FEED CONTAINING GLUTATHIONE AND VITAMIN E

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Abstract: This research is needed to accelerate spawning by adding glutathione and vitamin E in the feed to produce quality fish seeds. The aim is to determine the effect of adding vitamin E to commercial feed containing glutathione on increasing gouramy spawning. All treatment was carried out for two months using a Completely Randomised Design (CRD) with 4 treatments and 3 replications. Treatment A (glutathione), B (glutathione and vitamin E 150 mg.kg^{-1}), C (glutathione and vitamin E 300 mg.kg^{-1}), and D (glutathione and vitamin E 450 mg.kg^{-1}). 12 broodstock with male:female ratio of 1:1, weight range of 90-130 g obtained from nature. They fed as much as 5% of the fish body weight thrice daily. The results showed that the highest hatching rate was 99.67% in treatment D. The egg diameter in treatment C showed the best results (0.88 mm). The highest fecundity was 21.257 g in treatment D. The highest survival was 95.56% in treatment D. All feed treatments with a combination of glutathione and vitamin E gave a better response than the addition of glutathione alone. Polynomial regression analysis of the egg diameter parameter showed that the optimal dose of vitamin E was 282 mg.kg^{-1}.

Keywords: Glutathione, vitamin E, kissing gourami, spawning quality.

Introduction

Tambakan, or kissing Gourami is a potential freshwater fish for cultivation. The supply of fish ponds that rely on catches in nature cannot meet the needs of the market continuously because in the rainy season, fish in freshwaters moved, so fewer catches (Fitriliyani *et al*., 2022). Kissing Gurami is included in the blackfish group, it is often found in the swamp waters of Kalimantan and is the main catch of local fishermen because of its high price on the market (Ubamnata *et al*., 2015). In general, the people of South Kalimantan are more familiar with Gourami, which is the name of the Biawan fish.

The key to successful cultivation is the continuity of the availability of fry. This can be overcome by conducting artificial spawning (Raharjo *et al*., 2016) to mass-produce Gourami. Efforts to obtain optimal fish hatchery results include improving reproductive performance by improving the quality of parent/broodstock nutrition. In this study, the approach to the Broodstock was adding glutathione and vitamin E.

Glutathione is an antioxidant sulfhydryl (-SH), antitoxin, and enzyme cofactor that neutralises free radicals. Glutathione peroxidase (GPO) is an antioxidant enzyme that mediates oxidative damage (Kidd., 1997). Glutathione makes DNA or the building blocks of proteins and cells, supports immune function, forms sperm cells, breaks down some free radicals, helps the function of certain enzymes, and regenerates vitamins C and E (Haryanti *et al*., 2017). The use of Glutathione as a supplement in fish feed is reported to increase growth performance, such as in Chinese mitten crab (*Eriocheir sinensis*) (Liu e*t al.*, 2020) and white shrimp (*Litopenaeus vannamei*) (Xia & Wu, 2018). Schauer *et al.* (2004) reported that glutathione plays a role in free radical reduction, detoxification, nutrient

absorption, cell growth, cellular immunity, and DNA biosynthesis. Glutathione supplementation in feed can significantly improve growth performance and non-specific immunity as well as increase immune response and resistance to environmental stress (Xu *et al*., 2011; Ming *et al*., 2015; Ming *et al*., 2018; Xia & Wu, 2018).

Vitamin E has an important and decisive role in the reproduction of fish because vitamin E serves as an antioxidant that can prevent the oxidation of unsaturated fatty acids in cells. Vitamin E can protect fats from oxidising, such as fats or fatty acids found in cell membranes, so that the embryogenesis process runs normally and reproductive results can be improved (Napitu *et al*., 2013). Vitamin E is one of the important micronutrients that affect the reproductive performance of fish. Vitamin E in feed can increase spawning success, fecundity, hatching rate, survival rate, somatic gonad index, and vitellogenesis (Gammanpila *et al*., 2007). The role of vitamin E as an antioxidant is closely related to the mineral element selenium and the enzyme glutathione peroxidase (Lehninger., 2003).

The results of previous studies with the provision of vitamin E (α-tocopherol) mixed into feed ingredients have been shown to increase the reproductive potential of fish, such as in Hoven's carp fish (*Leptobarbus hoevenii*) the best dose of vitamin E is 300 mg.kg^{-1} feed (Aryani., 2007) and in Gourami fish the best dose of 338.72 mg.kg^{-1} feed (Basri., 2002). Arfah *et al.* (2013) reported that Vitamin E as much as 375 mg.kg^{-1} in feed can accelerate the maturation of gonads and increase fecundity, GSI, and egg diameter in Comet fish (*Carassius auratus*).

The purpose of this study is to examine the effectiveness of vitamin E addition to commercial feeds that contain glutathione in increasing spawning quality, and to determine the optimal dose of kissing Gourami, including hatching rates, egg diameters, fecundity, and survival rates.

Material and Methods

The Broodstocks Culture

Broodstocks was collected from the Martapura River, South Kalimantan Province. Fish were cultured in ponds in nine nets measuring 1 x 1 x 1 m and a water depth of 1.5 m for an adaptation period. The research was conducted from November 10, 2021 until January 10, 2022 at the Mentaos Freshwater Aquaculture Production Technical Implementation Unit, Banjarbaru City, South Kalimantan. The research design was completely randomised (CRD) with 4 treatments and 3 replications. The preparation of the rearing area for spawning was carried out by preparing 12 aquariums measuring 60 x 40 x 50 cm and 12 broodstock with male:female ratio of 1:1. Broodstocks weight range of 90-130 g obtained from nature (age less than 1 year). The water used for raising fish comes from wells with a temperature of 23-24^{o}C, and a pH of 5-7 and a DO of around 4-5 mg.L^{-1}. The edge of the aquarium is covered with black plastic so that the fish are not easily stressed. Water hyachint (*Eichhornia crassipes*) aquatic plants cover 10% of the surface for protection and are also useful for the easy adaptation of fish.

Application of Additional Supplements

The feed used is commercial feed (CF) with 30% protein, 8.75% fat, 11.5% moisture, and 6.5% Ash. Feed is given daily at 5% of fish biomass. All treatments contained glutathione with the same content (100 mg.kg^{-1}) but different levels of vitamin E. There were 4 treatments and 3 replications. Treatment A: Commercial feed + vegetable oil, glutathione, and egg white (Basic Feed/BF); treatment B: BF + vitamin E 150 mg.kg^{-1}; treatment C: BF + vitamin E 300 mg.kg^{-1} and treatment D: BF +, vitamin E 450 mg.kg^{-1}. The supplement was dissolved in 150 mL of coconut oil and stirred until homogeneous. The mixture was sprayed evenly onto the feed tested and then air-dried. The test feed sprayed with vitamin E at the

specified dose will be sprayed with egg white using a sprayer. The function of egg white is to coat commercial feed, given that vitamin E is an adhesive material added to commercial feed. Pellets were given to the test fish thrice daily, namely every morning, afternoon, and evening. The fish used for spawning are of the Gonad Maturation Level (GML) III - IV type (Nagahama *et al.*, 2008) with a minimum weight of 90-130 grams. Fish ponds were put into the rearing medium for 30 days according to the treatment. Females with mature genitals can be seen with the following characteristics: They are relatively thick, somewhat round, and have slow movements. The scales, especially from chin to belly, are whiter than males. The belly expands with the base of the pectoral fins reddish and greenish on the back while the belly is pale yellow. The male body is relatively thinner, elongated and looks agile. After being acclimatised in the culture vessel for 24 hours, the female broodstock was then spawned with the stimulating hormone ovaprim which was diluted using distilled water in a 1:1 ratio. Each treatment was injected with ovaprim at 0.5 $mL.kg^{-1}$ dose.

Larvae Maintenance

The fish eggs will hatch at intervals of 24 hours, then the larvae are left for 4 days until the egg yolk runs out. After that, the larvae were fed with *Artemia sp* four times daily at 07:00 a.m., 10:00 a.m., 14:00 p.m. and 17:00 p.m. ad libitum (Agustina *et al*., 2015). Feed is given to the larvae from 3-16 days old. The larvae were reared in a 19 × 18 × 18 cm jar and 15 cm of water with a stocking density of 6 fish/L (Joko *et al*., 2013). Water quality control is carried out by sucking up the dirt at the bottom of the aquarium and removing ± 30% of the water. Then, refill the wasted water with water from the reservoir.

Data Collection

The parameters of fecundity, egg diameter, hatching rate and larval survival rate were measured. All females with Gonad Maturation Level/GML III and IV were taken, their fecundity was calculated, and the eggs' diameter was measured. The fecundity calculation was done by removing GML IV eggs from fish stomachs. Then, the eggs were taken from the posterior, anterior, and middle gonads and then gonad sample was weighed (gonad sample weight). After that, it was put into a bottle and diluted with 10 mL of water. Then, 1 mL of the dilution was taken using a dropper followed by eggs were counted. Fish egg fecundity was calculated using a combined method, namely gravimetric and volumetric (Effendie, 2002).

$$F = \frac{G \text{ x } X \text{ x } V}{Q}$$

where:
F = fecundity (eggs)
G = gonado weight (gram)
Q = sample eggs weight (mm)
V = dilution volume (mm)
X = the sum of eggs in 1 cc

The diameter of fish eggs was observed by taking gonad fish samples with GML III and IV. Then, three parts were taken in the fish gonads, namely the posterior, anterior, and middle gonads, each part of 30 points and a total of 90. Fish egg samples were measured for diameter using an ocular micrometre (0.01 mm) brand UYCP-12. Fertilisation rate: Number of fertilised eggs/total eggs × 100 (Brommage and Cumalantunga, 1998). Hatching rate = (number of healthy fertilised eggs/number of fertilised eggs) × 100 (Hanjavanit *et al*., 2008). Survival rates were calculated during initial feeding according to the following formulae: SR = (No-Nt)/No, where SR = survival rate (%), Nt = total fish died during the experiment, No = total fish at the start of the experiment.

Correlation between parameters was analysed to assess the effect of the combination of supplementary feed containing glutathione with different levels of vitamin E. Those data were statistically analysed with Analysis of Variance (ANOVA) and polynomial orthogonal using SPSS program to determine the effect of supplementation on hatching rate, ovarian fecundity and egg diameter.

Results

Parameter data observed during this research is presented in Table 1.

The Egg Diameter

The results of the analysis of variance (ANOVA) show that the F count is 10.681 > F Table 5%, (4.07) which means that H_0 is rejected and H_1 is accepted, meaning that there is an influence between treatments on the egg diameter. The average diameter of eggs ranged from 0.76 to 0.88 mm, where the best egg diameter was produced at treatment C, which is 0.88 mm, then followed by treatment d of 0.85 mm, B of 0.80 mm, and the lowest at treatment A of 0.76 mm. By looking at the coefficient of variation (CV), which is 3.35%, the Honestly Significant Difference (HSD) or Tukey test will be continued. From the results of the Tukey test, treatment A is not significantly different from treatment B, but significantly different from treatment C and D. Treatment B is not significantly different from treatment D, but significantly different from treatment C. Treatment C does not differ significantly from treatment D.

The relationship of commercial feed with glutathione and vitamin E is different in the egg diameter of kissing fish, which can be seen in Figure 1 through simple linear regression and Figure 2 through polynomial regression.

The relationship between glutathione and vitamin E dose in commercial feed with fish egg diameter resulted in simple linear regression y = 0.00231 x + 0.7697, which shows that the egg diameter will increase along with the increasing dose of vitamin E in the feed. The value of R2 shows that the administration of glutathione and vitamin E in commercial feed influences the egg diameter of kissing fish by 74.18%, while

Table 1: Data of parameters

Parameters	Treatment			
	A	B	C	D
The egg diameter	0.76 ± 0,01^{c}	0.80 ± 0.04^{bc}	99.39 ± 0,576^{ab}	0.85 ± 0.03^{ab}
The hatching rate	98.39 ± 0,353^{b}	98.53 ± 0.288^{b}	99.39 ± 0,576^{ab}	99.67 ± 0.231^{a}
Fecundity	16.605,3 ± 1,638^{a}	17.932,0 ± 6.120^{a}	18.704,3 ± 2,300^{ab}	21.257,6 ± 1.520^{b}
Survival rate	88.89 ± 1,923^{b}	90.00 ± 3.330^{ab}	92.22 ± 1,923^{ab}	95.56 ± 1.928^{a}

Description: Numbers followed by letters that are not the same differ very significantly at the level of 5% Tukey Test (p > 0.05).

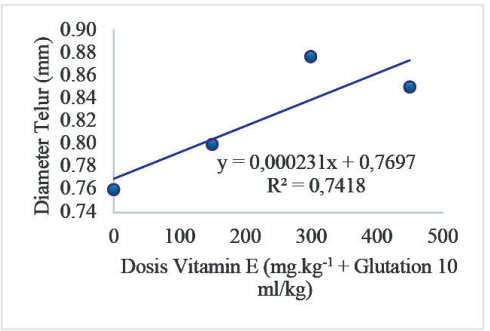

Figure 1: Simple linear regression

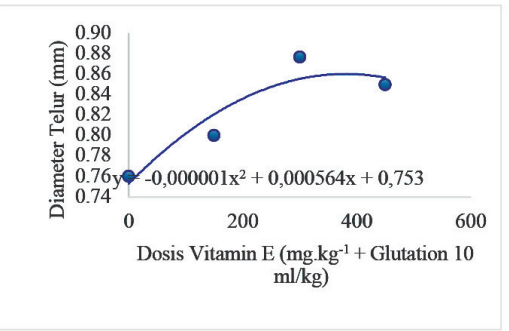

Figure 2: Polynomial regression

other factors influence the rest. In polynominal regression ($y = -0.000001x^2 + 0.000564x + 0.753$), the optimal dose of vitamin E can be calculated using the derivative equation, which is 282 $mg.kg^{-1}$.

Hatcing Rate

The hatching rate is the percentage of hatching ability of eggs fertilised by sperm to hatch (Murtidjo, 2001). The results of the analysis of variance (ANOVA) show that the F count is 8.053 > F Table 5%, (4.07) meaning that there is an influence between treatments on the hatching rate of kissing fish. The average hatching (HR) of kissing fish eggs ranged between 98.39-99.67%, where the best hatching degree was produced in the D treatment, which was 99.67%, then followed by the C treatment of 99.39%, B of 98.53% and the lowest in the treatment A of 98.39%. By looking at the coefficient of variation (CV), which is 3.87%, the Honestly Significant Difference (HSD) or Tukey test will be continued. From the results of the Tukey test, results showed that the treatment of A and B is not significantly different from the treatment of C, but significantly different from the treatment of D. The treatment of C and D does not differ markedly.

Fecundity

Fecundity is the number of mature eggs in the ovaries that are released for spawning. Based on fecundity, the number of fish produced can be estimated.

The results of the analysis of variance (ANOVA) show that the F count is 4.314 > F Table 5% (4.07), which means that H0 is rejected and accepted by H1, meaning that there is an influence between treatments on the fecundity of kissing fish. The average fecundity of kissing fish ranged between 16.605-21.257 g, where the highest fecundity was produced in the D treatment of 21.257 g, then followed by the C treatment of 18.704 g, B treatment of 17.932 g while the lowest was produced in the A treatment, which is 16.605 g. By looking at the coefficient of variation (CV), which is 9.2%, the least significant difference (LSD) or Fisher Test will be followed. The results of the Fisher test show that treatments A and B are not significantly different from treatment C, but significantly different from treatment D. Treatment C does not differ significantly from treatment D.

Survival Rate

The survival rate is expressed as the percentage of fish living during the maintenance period divided by the number stocked [9].

The results of the analysis of variance (ANOVA) showed that F count was 4,67 > F table 5%, (4,07) which means that hypothesis 0 was rejected and Hypothesis 1 was accepted, meaning that there was an influence between the treatments on the survival of pond larvae. The average survival rate ranged from 88.89-95.56%, where the best results were found in treatment D, namely 95.56%, then followed by treatment C at 92.22%, B at 90.00% and the lowest in treatment A at 88.89%. The coefficient of variation (CV) figure, which is 2.57%, will be followed by the Honest Significant Difference (HSD) or Tukey test. From the Tukey test, the results of treatment A were not significantly different from treatments B and C, but significantly different from treatment D. Treatment D was not significantly different from treatments B and C.

Water Quality

Water quality is a limiting factor for aquatic animals, both chemical, biological, and physical, so water quality parameters were measured in this study. Temperature, Dissolved Oxygen (DO) and pH measurements were carried out at the beginning of the study and were carried out several times when extreme weather changed and during the last spawning period. The results of observations of water quality at the beginning of the study and the average value during several sampling times during the maintenance period can be seen in Table 2.

Table 2: Water quality measurement results

Treatments	Temperature (°C)		DO (mg.L^{-1})		pH	
	Beginning	Average Value	Beginning	Average Value	Beginning	Average Value
A	28.00	31.00	4.50	6.30	7.60	7.50
B	30.07	31.33	4.53	7.47	7.62	7.58
C	29.27	30.37	3.87	7.17	7.50	7.51
D	28.57	30.10	3.40	7.60	7.63	7.52

Discussion

In this study, feed treatments containing the same content of glutathione (100 mg.kg^{-1}) but different levels of vitamin E showed different responses, where treatment C (300 mg.kg^{-1} vitamin E) showed the highest egg diameter response compared to other treatments. Meanwhile, treatment D (450 mg.kg^{-1} vitamin E) produced the highest fecundity compared to other treatments. While it was seen that all treatments that combined glutathione with vitamin E (treatments B, C and D) showed a better response compared to treatment A, which only used additional glutathione without a combination of vitamin E.

Vitamin E, which is given in broodstock feed has an important role in the reproductive process, which ultimately affects egg quality, hatchability, and survival and functions as a balancer in cell metabolism as an intracellular anti-oxidant (Santiago & Gonzac, 2000; Palace & Werner, 2006). The relationship between vitamin E and the development of egg diameter through the mechanism of action of prostaglandins synthesised enzymatically using essential fatty acids is related to the role of vitamin E as an antioxidant (Yulfiperius & Jusadi, 2003). If the egg lacks essential fatty acids, then the process will fail, resulting in a low hatching rate (Mokogintam *et al*., 2004). The results of this experiment show that there is a relationship between the content of vitamin E in feed and the value of egg diameter, which is produced at treatment C (glutathione + 300 mg.kg^{-1}), which is 0.88 mm, then followed by treatment D (glutathione + 450 mg.kg^{-1}) of 0.85 mm, B (glutathione + 150 mg.kg^{-1}) of 0.80 mm, and the lowest at treatment A (only glutathione) of 0.76 mm. The results were compared with Mizan *et al.* (2018), kissing gouramy who were given additional turmeric powder into the feed produced egg diameters ranging from 0.74-0.90 mm. Sink and Lochmann (2008) reported that an increase in egg diameter indicates oocyte development, where oocyte development occurs due to the accumulation of egg yolk. Yolk accumulation consists of two phases: Endogenous vitellogenesis (synthesis of yolk within the oocyte) and exogenous (accumulation of yolk precursors synthesised outside the oocyte). Larvae will have a greater chance of survival than smaller egg sizes (Tyler & Sumpter, 1996). Larvae that hatch will depend on food reserves (yolk bag) which are stored until they enter the stage of opening their mouths to start eating food intake from outside and after that, the chemical components in the eggs will be separated from the larvae (Watanabe, 1994). The increase in egg yolk volume is also due to the high increase in vitellogenin in the ovary during the vitellogenesis process where it affects the value of egg diameter in fish (Nagahama & Yasmita, 2008; Utomo, 2009).

The content of vitamin E affects the reproductive rate of fish, vitamins are organic compounds that are important for the growth, reproduction and health of fish as well as a metabolic booster in the fish's body so that the feed given to broodfish containing vitamin E will affect reproduction and metabolic rate (Shahkar *et al*., 2018). Additives added to feed

can help stimulate the vitellogenesis process, as reported by Garcia and Lara (2013), turmeric contains phytosterols, carotenes, vitamin E, and curcumin which resemble phytoestrogens and hepatoprotection from 27 groups of flavonoids which can act as estrogens that stimulate heart function to synthesise vitellogenin.

With the help of regression calculations, the egg diameter will increase along with the increasing dose of vitamin E in the feed. The optimal dose of vitamin E through the derivative equation is obtained, which is 282 mg.kg^{-1}. This value indicates that an increase in Vitamin E exceeding 282 mg.kg^{-1} will not be effective in increasing the diameter of fish eggs. The diameter of the egg will affect the availability of egg yolk, where the larger the egg diameter, the more egg yolk supply will be. This data is supported by the value of hatchability, where hatchability in treatment D (300 mg.kg^{-1}) reached 99%, which was higher than that of the other treatments. Good egg quality can be seen from a high hatching rate (Palace & Sullivan, 2002; Mansouret *et al.*, 2006). Previous research from Serezliet *et al*. (2010) found that vitamin E is an antioxidant and can improve sperm and ovum quality, egg fertility and affect gamete quality. The fertilisation factor is largely determined by how many egg cells can be fertilised by sperm. The more egg cells fertilised by sperm, the higher the hatchability and vice versa, unless environmental factors influence it and genetic factors (Masrizal & Efrizal, 1997; Sariet *et al*., 2002). Izquierdoet *et al*. (2001) reported that vitamin E can provide an important protective role for sperm cells during spermatogenesis and fertilisation to prevent the risk of lipid peroxidation.

Kidd (1997) reported that the role of glutathione can maximise the work of vitamin E in the spawning process to produce eggs and quality larvae with a good immune system. Xue *et al.* (2022) reported that the glutathione treatment showed higher daily growth, body weight gain and protein efficiency ratio and lower feed conversion than the control treatment without glutathione. The mechanism of the growth promoter may be related to the structure of the glutathione molecule, which is a tripeptide containing γ-amide bonds and a sulfhydryl group, which is composed of glutamic acid, cysteine, and glycine. Cysteine is a component of coenzyme A, which can break the disulfide bonds of somatostatin molecules, relieve somatostatin inhibition on growth hormones and promote growth (Deneke & Fanburg, 1989; Lafleur *et al*., 1994; Kidd, 1997; Ming *et al*., 2015). Glutathione can also increase Triiodothyronine (T3) content and stimulate insulin-like growth factor (IGF)-1 in carp blood serum (Ming *et al*., 2015; Xue *et al.,* 2022). In this study, the combination of 100 mg.kg^{-1} of glutathione with 450 mg.kg^{-1} of vitamin E produced the highest fecundity and was different from treatments A and B but not from treatment C. This is presumably because the combination of glutathione and vitamin E content plays an important role in improving the immune system, body metabolism, and increases the ability to deal with stress, providing the ability to activate growth so that the process of vitellogenesis that occurs in the liver is more optimal (Deneke & Fanburg, 1989; Lafleur *et al*., 1994; Kidd, 1997; Ubilla & Valdebenito, 2011; Ming *et al.,* 2015; Shuqun Xueet *et al.,* 2022). This opinion is supported by Fahriny and Syarifuddin (2010), each individual in one fish species has a different number of eggs, one of which is influenced by the quality of the feed consumed and its ability to deal with environmental conditions.

According to Aryani and Alawi (2014), an increase in the value of fecundity will be in line with the high vitamin E content in the feed. This statement is supported by Napitu *et al*. (2013), who state that increasing the absorption of vitellogenin by the ovaries in the reproductive phase will result in the formation of several eggs in the ovaries. Batubara (2009) said that the quantity and quality of feed such as protein, fat, and vitamins given to the broodstock is an important factor closely related to gonad maturity, the number of eggs produced, and egg production and larval quality. Palace and Werner (2006) reported that environmental factors influenced the increase in the value of fecundity,

the quality of the sires and the amount of feed nutrients, as well as the efficiency of feed utilisation. This was allegedly due to the factor of energy allocation for the body's balance process so that energy was used to form eggs which increased fecundity value.

Sukendi *et al*. (2013) reported that the carp (*Trichogaster pectoralis*) produced a fecundity of 15,105 eggs with the addition of 298 mg.kg^{-1} of vitamin E. Fitriliyani *et al*. (2022) reported that artificial feed with the addition of a fortified combination of glutathione and vitamin E with a composition content of 300 mg-700 mg.kg^{-1} of feed had a better effect on increasing the gonadosomatic index (GSI), hepatosomatic index (HSI).

The beginning and average values during this study ranged from 28, 00-31, 33°C, and the results obtained from the study are still within normal limits to support the process of gonad maturation and spawning and survival rate. Temperature affects the levels of dissolved oxygen in the water, the higher the water temperature, the faster the water will experience oxygen saturation (Effendi, 2009). According to Yurisman (2009), the optimum temperature for the growth of tambakan is between 25-30°C. The temperature condition in this study is slightly higher than the optimum temperature proposed by Yurisman (2009). The results of the measurement of dissolved oxygen (DO) early and late in this study ranged between 3.4-7.6 mg.L^{-1}, from the results of this study, is still included in the normal range to support the process of maturation of gonads and spawning and survival rate. Hidayat (2013) states that dissolved oxygen content plays an important role in the waters. For fish life, dissolved oxygen is not required to be less than 2 mg L^{-1} or at least 1.7 mg L^{-1}. The life of the fish can be said to be viable and successful, so the dissolved oxygen content should not be less than 4 mg.L^{-1}. The pH measurement results in this study ranged from 7.5 to 7.63. According to Effendi (2009), good water for fish farming is in a neutral range with a pH of 7.0-8.0. The acidity (pH) expresses the hydrogen ions (H^+) concentration in water. The amount is expressed minus the logarithm of the concentration of H ions, pH indicates the strength between acid and wet in water. According to Hidayat (2013), the pH suitable for freshwater fish ranges from 6.7 to 8.6.

Conclusions

This study gave interesting results, where the feed with a combination of 100 mg.kg1 glutathione and vitamin E 150, 300, and 450 mg.kg^{-1} gave a better response to increased hatchability, egg diameter, fecundity, and survival compared to feeding only with the addition of glutathione alone. Polynomial regression analysis of the egg diameter parameter showed that the optimal dose of vitamin E was 282 mg.kg^{-1}.

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Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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