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ORIGINAL RESEARCH

# Optimizing the gonadal performance of broodstock *Helostoma temminckii* with the addition of glutathione and vitamin E as enrichment ingredients in feed

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***Abstract.*** Vitamin E and glutathione are antioxidants that play a role in reducing Reactive oxygen species that occur when an organism experiences stress and it's plays a role in membrane permeability, especially for the stages of fish gonad development. The purpose of this research was to optimizing the gonadal performance of broodstock kissing gouramy *Helostoma temminckii* with the addition of glutathione and vitamin E as enrichment ingredients in feed so as to improve the quality and quantity of seeds produced. Fish were randomly allocated into four treatments were Treatment A=Commercial feed added Glutathione, B=Commercial feed added with Glutathione and Vitamin E 300 mg/Kg; C=commercial feed added with glutathione and vitamin E 500 mg/Kg and D=commercial feed added with glutathione and vitamin E 700 mg/Kg; using three replicates for each treatment (18 fish/treatment, 6 fish/replicate), the experiment lasted for 30 days. The rearing container used in the study were 12 hapa measuring 1 x 1 meter placed in the pond with a water level of 50 cm. The results showed that all treatments with the addition of glutathione and vitamin E fortifications with levels of 300 mg-700 mg gave the effect of increasing Gonadosomatix Indeks (GSI), Hepatosomatic Indeks (HSI), higher fecundity and larger egg diameter than treatment A. The amount of initial GSI increase in treatment A was 33.49%, treatment B was 172.52%, treatment C was 208.60% and treatment D was 691.20%. While the HSI value at the end of the study decreased in treatment A by 35.30% and treatment C which also experienced a decrease in HSI by 26.23%. While in treatments B and D there was a not too large increase, in the range of 42.56% (B) and 5.66% (D). The artificial feed with addition combination of glutathione and vitamin E fortifications with levels of 300 mg-700 mg, gave a better effect to increase Gonadosomatix Indeks (GSI), Hepatosomatic Indeks (HSI), and higher fecundity and larger egg diameter than compared with single glutathione treatment without combination with vitamin E.

**Keywords**: enrichment, feed, glutathione, vitamin E, gonads

## INTRODUCTION

Kissing gourami fish more often called biawan fish in Indonesia (*Helostoma temminckii*) is a native Indonesian fish found in several rivers in Sumatra and Kalimantan. Such as the areas of Nanggroe Aceh Darussalam, North Sumatra, West Sumatra, Riau, West Kalimantan, Central Kalimantan, South Kalimantan and East Kalimantan. These fish live in rivers, creeks and inundation areas upstream to downstream, even in estuaries of rivers that are muddy and forested on their sides. This fish has high economic value, has aquaculture development prospects with great opportunities, the selling price is quite expensive, is an important commodity in the freshwater fish business but this fish is still rarely cultivated until now [1].

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Kissing gourami fish is very potential to be cultivated because it has several advantages such as high adaptability to waters with low dissolved oxygen levels and belongs to a group of fish that have high fecundity values [2]. Kissing gourami fish production currently still depends on the catch. in nature, while for maintenance in a controlled container has not been done by many farmers [3]. Given this, the Kissing gourami fish has great potential to be cultivated.

The problem in the development of Kissing gourami fish farming is quality seeds that cannot be continuously available. This is because one of the reasons is that the gonad maturity of the broodfish parent when reared in a culture environment is different from that when the kissing gourami broodstock is large in its original culture environment. The

decrease in the carrying capacity of the aquatic environment is also the cause of the decline in the quality of fish gonads. Vitamin E and glutathione are antioxidants that play a role in reducing Reactive oxygen species (ROS) that occur when an organism experiences stress and it's plays a role in membrane permeability, especially essensiall fatty acid regulation for the stages of fish gonad development.

At this time, feed manufacturers are still unable to accelerate in increasing gonadal maturity, so it is necessary to have additional ingredients in the form of hormonal additions and supplement materials in feed that can accelerate the gonad maturation of Kissing gourami fish.

Administration of vitamin E mixed in the feed at different doses for nile tilapia had a significant effect on the achievement of gonad maturity level, gonadosomatic index (GSI), and Fecundity ($P < 0.05$). Provision of vitamin E at a dose of 300 mg/kg of feed was the best dose (31) Previous reports showed that the addition of vitamin E in feed was successfully improved the egg production and quality of *Osteochilus kelabau* [4], *Oreocromis niloticus* [5], *Carrassius auratus* [6]; *Osteochilus hasselti* [7]; and *Oxyeleotris mamorata* [8].

Vitamin E has a very important role in increasing fish reproduction because vitamin E functions as an antioxidant that can maintain the presence of fatty acids and prevent fat oxidation in cell membranes and can accelerate the secretion of reproductive hormones.

The maintenance of papuyu broodstoc was carried with commercial feed mixed with 10 ml / kg of Beta glucan and Vitamin E 150 mg / kg of feed for treatment had a significant effect on the percentage of gonado somatic index (GSI), but had no significant effect on the hepatosomatic index (HSI).

Glutathione is another a natural antioxidant consisting of three amino acids namely cysteine, glutamate, and glycine. These three types are essential amino acids that are essential for growth. There are various roles of glutathione in the work of the body's chemical reactions, for example helping to remove toxins, including those from the body, the drugs consumed, or the environment. As an antioxidant, glutathione is important for controlling the number of free radicals which usually increase with increasing environmental stress. [9] This study aims to optimize the gonads of kissing gouramy fish by giving artificial feed enriched with combination glutathione and vitamin E, so as to improve the quality and quantity of seeds produced.

# METHODOLOGY

## Broodstock Maintenance

The broodstock used in this study came from the Bincau Freshwater Fish Hatchery Center, South Kalimantan. Fish were randomly allocated into four treatments whose commercial diets were supplemented with single gluthatione and three levels combination with different level vitamin E.

respectively, using three replicates for each treatment (18 fish/treatment, 6 fish/replicate), The experiment lasted for 30 days. The rearing container used in the study were 12 hapa measuring 1 x 1 m placed in the pond with a water level of 50 cm.

One teaspoon of vegetable oil is used as a solvent for vitamin E and glutathione, while one teaspoon of egg white is used as an adhesive. This mixture is dissolved in 100 ml of alcohol and sprayed on commercial feed.

Maintenance of brood fish during the 30-day study by giving commercial feed (CP PRIMA FEED), Vitamin e (Ovagrog vit e) and Gluthatione (L Gluthatione 300mg/capsul by SQUARE), Giving continuously 3 times a day at 07:00, 12:00 and 17:00 with a normal dose 3% of the total weight of the fish.

## Treatment

The glutathione used in this study was 10 ml/kg of feed. The eggs used were 2-3 egg whites, which used to coat the feed when it has been processed and dried. The treatments that will be given are as follows: The experimental design used in this study was a completely randomized design (CRD). The total number of experimental units was 12 units, with 4 treatments and 3 replicated. The treatment is:

Treatment A = Commercial feed (CF) added with, glutathione (G)
Treatment B = Commercial feed added with glutathione + Vitamin E 300 mg/kg
Treatment C = Commercial feed added with glutathione + Vitamin E 500 mg/kg, ,
Treatment D = Commercial feed added with glutathione Vitamin E 700 mg/kg

## Parameter Measurement

Gonad and liver samples of fish were taken to dissect the fish in each treatment. Gonadosomatic index (GSI) is the calculation of the gonad mass as a proportion of the total body mass. The GSI propose is as an alternative way to estimate the gonad maturity. It is presented by the formula.

According to [10], the calculation of the Gonadosomatic index (GSI) is calculated by the Gravimetric method, using the following formula:

$$GSI = \frac{Wg}{Wt} \times 100\%$$

Information:
Wg = Fish Gonad Weight (g)
Wt = Body Weight of Test Fish (g)

**Hepatosomatic Index (HSI)**
Hepatosomatic Index (HSI) is a quantitative value that can describe the increase in liver weight along with gonadal development and an increase in the gonadosomatic index GSI [11]. According to [10], the calculation of the hepatosomatic index (HSI) is calculated by the Gravimetric method, using the following formula:

$$HSI = \frac{Wh}{Wi} \times 100\%$$

Information:
Wh = Fish Liver Weight (g)
Wi = Body Weight of Test Fish (g)

**Egg diameter**
Measurement of the egg size was done by measuring the egg diameter in millimeter (mm) using KEYENCE digital microscope (VHX-500). For each female fish, fifteen eggs were collected from each of the anterior, middle and posterior region of the ovary and the diameter measured on position horizontal and vertical.

**Maintenance Water Quality**
Water quality in this study observed several parameters, including: dissolved oxygen (DO), acidity (pH), temperature, and ammonia (NH3). Sampling of water quality was carried out twice, namely at the beginning and at the end of the study.

**Statistical Analysis**
Gonadosomatic Index (GSI) and Hepatosomatic Index (HSI), egg diameter, and fecundity were expressed as mean ± SD and were analyzed statistically using analysis of variance (ANOVA) using the SPSS version 26 program with Duncan's test.

## RESULTS AND DISCUSSION

### Gonadosomatic Index (GSI) and Hepatosomatic Index (HSI)

The data for each treatment are presented in Table 1 below for the calculation of the initial GSI and HSI data. The data above was the initial data for the calculation of GSI and HSI, while the calculation of GSI and HSI at the end of the study was presented in Table 2 below.

**Fecundity**
Egg fecundity data at the beginning and end of the study are presented in Table 3 below.

**Egg Diameter**
The data egg diameter obtained are presented in Table 4 below.

**Table 1.** Calculation of Gonadosomatic Index and Hepatosomatic Index at the beginning of the study for each treatment unit

| Treatment | Initial Fish Weight (g) | Initial Gonad Weight (g) | Initial Liver Weight (g) | Initial GSI (%) | Initial HSI (%) |
|---|---|---|---|---|---|
| A | 145.67 ± 13.05 | 9.59 ± 3.14 | 1.75 ± 0,49 | 6.49 ± 1.6 | 1.19 ± 0.26 |
| B | 84.33 ± 4.93 | 3.13 ± 2.11 | 0.55 ± 0,19 | 3.63 ± 2.22 | 0.65 ± 0.21 |
| C | 82.67 ± 6.43 | 2.64 ± 2.22 | 1.07 ± 0,57 | 3.07 ± 2.34 | 1.29 ± 0.69 |
| D | 83.33 ± 15.01 | 0.99 ± 0.63 | 0.69 ± 0,25 | 1.16 ± 0.71 | 0.82 ± 0.23 |

A : Commercial feed (CF) added, glutathione (G)
B : Commercial feed (CF) added , glutathione (G), Vitamin E 300 mg/kg
C : Commercial feed (CF) added , glutathione (G), Vitamin E 500 mg/kg
D : Commercial feed (CF) added glutathione (G), Vitamin E 700 mg/kg

**Table 2.** Gonadosomatic Index and Hepatosomatic Index Calculation Data at the end of each treatment unit

| Treatment | Final Fish Weight (g) | Final Gonad Weight (g) | Final Liver Weight (g) | Final GSI (%) | Final HSI (%) |
|---|---|---|---|---|---|
| A | 155.11 ± 10.49 | 13.69 ± 7.66 | 1.20 ± 0.13 | $8.66 \pm 4.51^{ab}$ | $0.77 \pm 0.07^{a}$ |
| B | 122.55 ± 15.25 | 11.91 ± 1.06 | 1.12 ± 0.02 | $9.89 \pm 2.15^{ab}$ | $0.93 \pm 0.11^{a}$ |
| C | 143.33 ± 23.63 | 13.52 ± 2.64 | 1.35 ± 0.10 | $9.48 \pm 1.40^{b}$ | $0.95 \pm 0.10^{a}$ |
| D | 121.61 ± 11.35 | 11.37 ± 5.75 | 1.07 ± 0.16 | $9.15 \pm 3.86^{a}$ | $0.73 \pm 0.13^{a}$ |

Note: Different letters on the same line show significant difference affects (P< 0.05). The values listed are mean ± SE.

**Table 3.** Fecundity value per treatment on day 0 (beginning) and day 30 (end)

| Observation time | Fecundity Value per Treatment (Item) | | | |
|---|---|---|---|---|
| | A | B | C | D |
| Beginning of research | 13,557.33 ± 4,334.98 | 5,013.33 ± 3,369.67 | 11,365.33 ± 8,253.90 | 1,912.00 ± 604.36 |
| The end of the research | 21,907.56 ± 12,253.41[a] | 19,056.00 ± 1,703.25[a] | 21,632.00 ± 4,216.68[a] | 18,197.33 ± 9,199.28[a] |

Note: Different letters on the same line show significant difference affects (P< 0.05). The values listed are mean ± SE.

**Table 4.** Kissing gourami Fish Egg Diameter Data During Research

| Observation time | Egg diameter (µm) for each treatment | | | |
|---|---|---|---|---|
| | **A** | **B** | **C** | **D** |
| Initial Horizontal | 1.250,54 ± 24,95 | 1.181,88 ± 99,57 | 1.132,38 ± 42,72 | 1.200,78 ± 45,88 |
| Final Horizontal | 1,251.86 ± 1.87[b] | 1,218.52 ± 51.81[b] | 1,175.94 ± 61.60[ab] | 1,189.36 ± 16.16[a] |
| Initial Vertical | 1.297,77 ± 29,60 | 1.210,10 ± 73,49 | 1.197,37 ± 17,40 | 1.257,19 ± 32,51 |
| Final Vertical | 1,292.18 ± 7.91[a] | 1,265.67 ± 78.59[a] | 1,215.57 ± 25.73[a] | 1,280.11 ± 32.41[a] |

Note: Different letters on the same line show significant difference affects (P< 0.05). The values listed are mean ± SE

Statistic analyzes in showed Final HSI value (Table 2) and fecundity (Table 3) and vertical fish egg diameter of the experiment on day 30 no significant difference. However GSI of treatment C (CF + G + Vit E 500 mg/kg) A (CF + G) and B (CF + G + Vit E 300 mg/kg) no significant difference too, but GSI on treatment C higher and significant difference with GSI on treatment D (CF + G + Vit E 700 mg/kg). Significant value was also showed in horizontal fish egg diameter, where treatment A and B significant difference with treatment D.

An interesting thing was shown when we look at the value of HSI and GSI beginning of the research (Table 1, 2 and Table 3) and calculated the percentage increase or decrease all parameters. The amount of initial GSI increase in treatment A was 33.49%, treatment B was 172.52%, treatment C was 208.60% and treatment D was 691.20%. While the HSI value at the end of the study decreased in treatment A by 35.30% and treatment C which also experienced a decrease in HSI by 26.23%. While in treatments B and D there was a not too large increase, in the range of 42.56% (B) and 5.66% (D). There was an increase in the number of eggs in all treatments, the largest was in treatment D (851.74%), treatment B (280.33 %), treatment C (90.33%) and treatment A the smallest of 61.59%.

Measurements of egg diameter in all treatments with the addition of feed additives showed egg diameter in treatment B (V 9.18%, H 6.20%), treatment C (V 3.04%, H 7.69%); and treatment D (V 3.65% H -1.90%). Compared with treatment A, the diameter of V decreased by 0.86% and H increased by only 0.21%.

In all treatments that were given a combination of glutathione and vitamin E (Treatments B, C and D) showed a higher GSI value than the treatment with feed A (control feed without additional feed additives). The amount of initial GSI increase in treatment A was 33.49%, treatment B was 172.52%, treatment C was 208.60% and treatment D was 691.20%. While the HSI value at the end of the study decreased in treatment A by 35.30% and treatment C which also experienced a decrease in HSI by 26.23%. While in treatments B and D there was a not too large increase, in the range of 42.56% (B) and 5.66% (D).

**Discussion**

From the data in Table 1, it was clear that compared to the beginning of the study all treatments showed an increase in GSI values. In all treatments that were given a combination of glutathione and vitamin E at treatments B (CF + G + Vit E 300 mg/kg), C (CF + G + Vit E 500 mg/kg) and D (CF + G + Vit E 700 mg/kg) showed a higher GSI value than the treatment with feed A (CF + G).

According to [12] there is an opposite mechanism between the GSI and HSI values. Where when the HSI value is high, the GSI value tends to be lower, otherwise the increase in the GSI value will be followed by a decrease in the performance of the HSI value. This is because the stages of improving the performance of HSI show an increase in the process of vitellogenesis in the liver and if vitellogenin as a source for the formation of

egg yolks has started to enter the stage of egg follicle development, the GSI will increase. The increase in GSI value was much more than doubled in the feed with the stimulation of the addition of glutathione and vitamin E in the feed [13].

The high GSI value in treatment D was thought to be because the specific mechanism of interaction glutathione and vitamin E. According to [14] an important line of defense is formed by antioxidants. Vitamin E (consisting of various forms of tocopherols and tocotrienols) is an important fat-soluble, chain-breaking antioxidant. Besides working as an antioxidant, this compound possesses other functions with possible physiological relevance. The glutathione-dependent enzymes form another line of defense. Two important enzymes in this class are the free radical reductase and glutathione S-transferases (GSTs). The GSTs are a family of phase II detoxification enzymes. They can catalyze glutathione conjugation with various electrophiles. In most cases the electrophiles are detoxified by this conjugation, but in some cases the electrophiles are activated. Antioxidants do not act in isolation but form an intricate network. It is, for instance, known that vitamin E, together with glutathione (GSH) and a membrane-bound heat labile GSH-dependent factor, presumably an enzyme, can prevent damaging effects of reactive oxygen species on polyunsaturated fatty acids in bio membranes (lipid peroxidation). It is well known that the fatty acid composition of the diets normally plays the pivotal roles in the gonadal development, immune performance, osmoregulation, and stress resistance [15, 16, 17]. According to [11], during the reproductive process, the energy produced by the body was mostly used for the development of the gonads. Fish gonad maturity occurs when the fish will spawn. At that time, the gonads will experience weight gain until it reaches a maximum and then will experience a decrease in weight after spawning occurs.

Stated that nutrition in broodstock feed can affect gonadal development, fecundity, and embryo development [18]. This was in line with the results of the study where the addition of enriching ingredients in feed such as glutathione and vitamin E resulted in the highest GSI among other treatments. According to [19], one of the nutrients that can be given to broodstock to improve reproductive performance and egg quality is vitamin E. Vitamin E or commonly called α-tocopherol has the main function as an antioxidant that prevents the peroxidation of polyunsaturated fatty acids (PUFA) from phospholipids and cholesterol in cell walls. PUFA is an essential nutrient found in fish eggs and plays an important role in the early development of fish. Furthermore, [20] stated that to prevent the process of fatty acid peroxidation, vitamin E can be used as a supplement in feed.

Vitamin E (α-tocopherol) is a fat-soluble vitamin that has a major role as an antioxidant, and is known to play a role in helping reproductive performance in fish. Vitamin E also functions as a protective cell wall from toxic materials such as lead, mercury, benzene, and free radicals that can interfere with the work of the endocrine glands and result in the balance of hormone production. Vitamin E given to brood fish will be digested in the small intestine and stored in several body tissues such as adipose tissue, liver, and other body tissues.

Vitamin E has a very important role in determining the reproduction of fish, the need for vitamin E for reproduction is different for each fish species [21]. Fish that are deficient in vitamin E can affect reproductive performance, cause gonad immaturity, low egg hatching rates, and seed survival [22]. The need for vitamin E can increase along with the increase in the amount of fatty acids in the feed. Vitamin E added to feed to accelerate the follicular formation phase. [23] states that the need for vitamin E in rainbow trout is 30 mg/kg feed, salmon fish 30 mg/kg feed, carp 80-100 mg/kg feed, American catfish 30 mg/kg feed, and red fish sea bream requires 42 mg/kg feed [24], milkfish 40 mg/kg feed [25].

Gonadic growth occurs when the energy used for somatic growth has been met [26]. In addition, vitamin E (α-tocopherol) is one of the nutrient elements that must be met in feed, because vitamin E is needed as a building block for somatic, gonadic structures, and determinants of egg quality. Vitamin E also functions as an antioxidant, so that unsaturated fatty acids in phospholipids in cell membranes are protected [27].

Fecundity describes the number of eggs in the egg bag. In this study, it was seen that there was an increase in fecundity during the 30 days of rearing the broodstock. All treatments with the addition of glutathione and vitamin E as feed additives gave the effect of increasing the number of eggs higher than treatment A.

According to [28], differences in the fecundity of a species and the same size of fish can occur because each has a different fat content. This is in line with the results of the study which showed that treatment with the addition of enriching agents resulted in a much more

significant increase in fecundity or the number of eggs compared to without the addition of enrichment agents.

Vitamin E has a very important and decisive role in fish reproduction, because vitamin E functions as an antioxidant that can prevent the oxidation of unsaturated fatty acids in cells [21]. As an antioxidant, vitamin E can protect fats from being oxidized, for example fats or fatty acids found in cell membranes, so that the embryogenesis process runs normally and reproductive results can be increased [21]. This research was in line with [28], which stated that vitamin E supplementation of 189.65 mg/kg of feed could improve the quality of catfish eggs. Furthermore, [29], also stated that vitamin E supplementation to levels of 300 mg/kg of feed tended to improve the reproductive quality of tilapia.

Fecundity is very important for the study of population dynamics and life history of fish [30]. In general, fecundity is defined as the number of eggs found in the female parent shortly before spawning [31]. The high value of fecundity in the treatment with the addition of enriching agents compared to the control treatment is thought to be due to the function of vitamin E which can prevent oxidation of fatty acids [32, 33], so that fat can be fully utilized for development. gonads and fecundity values can be increased. This is in line with the results of research by [29] regarding the effect of adding vitamin E in feed formulations showing an increase in the reproductive quality of tilapia, including the speed of achieving gonadal maturity, number of eggs produced (fecundity), egg weight, egg diameter, fertility, hatchability and total larvae produced, which was better than the yield of broodstock fed without the addition of vitamin E.

Measurement of egg diameter, which was carried out vertically (V) and horizontally (H) at the end of the study, showed a significant increase compared to the beginning of the study. Measurements in all treatments with the addition of feed additives, treatments B, C and D gave the percentage increase in egg diameter. Egg diameter has something to do with fecundity. The more eggs that are spawned, the smaller the diameter of the eggs, and vice versa [34]. It is also stated by [35] that fish with smaller egg diameter usually have more fecundity, while those with large egg diameter tend to have low fecundity. The larger the diameter of the eggs, the better, because the eggs are provided with food reserves so that the fish larvae will be able to survive longer. Larvae that come from large eggs have the advantage of having more yolk reserves as a source of energy before getting food from outside.

The size of the diameter of the egg can determine the quality associated with the content of the yolk. Large eggs can also produce large larvae. Stated that the more developed the gonads, the larger the diameter of the eggs in them as a result of yolk deposition, hydration, and the formation of oil droplets [11]. The addition of enriching ingredients in the form of glutathione and vitamin E is thought to be able to maintain the presence of fat granules, thereby increasing the number and size of egg yolk granules, resulting in an increase in egg volume and diameter [36]. As stated by [37], which stated that the addition of vitamin E to the feed can increase the size of the egg diameter of comet fish (*Carassius auratus auratus*). The egg must contain all the nutrients needed by the developing embryo and larva after the egg is fertilized so that the fish can meet their needs to initiate the consumption of external food. Large egg size is a guarantee of higher survival [38].

Vitamin E (consisting of various forms of tocopherols and tocotrienols) is an important fat-soluble, chain-breaking antioxidant. Besides working as an antioxidant, this compound possesses other functions with possible physiological relevance. The glutathione-dependent enzymes form another line of defense. Two important enzymes in this class are the free radical reductase and glutathione S-transferases (GSTs). The GSTs are a family of phase II detoxification enzymes. They can catalyze glutathione conjugation with various electrophiles. In most cases the electrophiles are detoxified by this conjugation, but in some cases the electrophiles are activated. Antioxidants do not act in isolation but form an intricate network. It is, for instance, known that vitamin E, together with glutathione (GSH) and a membrane-bound heat labile GSH-dependent factor, presumably an enzyme, can prevent damaging effects of reactive oxygen species on polyunsaturated fatty acids in bio membranes (lipid peroxidation) [39]. Recent evidence suggests that vitamin E enhances glutathione levels. Glutathione, another natural antioxidant, may also be important in blood pressure and glucose homeostasis [40].

This is in line with the results of this study, where the combination of glutathione and vitamin C yielded better HSI, GSI, fecundity and egg diameter values than the use of single glutathione.

## CONCLUSION

The artificial feed with addition combination of glutathione and vitamin E fortifications with levels arrange of 300 mg-700 mg, gave a better effect to increase gonadosomatic index (GSI), hepatosomatic index (HSI), and higher fecundity and larger egg diameter than compared with single glutathione treatment without combination with vitamin E.

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