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EFFECT OF THE VITAMIN E IN COMMERCIAL FEED CONTAINING GLUTATHIONE ON HISTOLOGY LIVER AND GONADS OF KISSING GOURAMI FISH

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ABSTRACT
Kissing gourami fish is one of the swamp fish in South Kalimantan. Vitamin E is a micronutrient with important role for reproduction, the addition vitamin E for gonad maturation in Kissing gourami fish still not done. This research aims to determine the optimal dose of Vitamin E in commercial feed containing glutathione for the liver and gonads of Kissing gourami fish and to determine the histology of the liver and gonads of Kissing gourami fish. This research was conducted at the Puja Kesuma fish pond, Sungai Sipai Irrigation, Martapura Sub-District, Banjar Regency, South Kalimantan Province, with a rearing time of ± 5 weeks. The design used in this research was a completely randomized design (CRD) with 4 treatments and 3 replications with the following treatments: A (Dose 0 mg/Kg Vitamin E), B (Dose 300 mg/Kg Vitamin E), C (Dose 500 mg/Kg), mg/Kg Vitamin E), D (Dose 700 mg/Kg Vitamin E). Research shows that vitamin E did not significantly affect the parameters of HSI, GSI, egg diameter, and fecundity, while the histological observations of the liver and gonads found the effect of vitamin E. In liver histology, liver cell damage was found, while in gonad histology, oocyte patterns were found in phases 1-4.

KEY WORDS
Kissing gourami fish, vitamin E, feed, histology, liver, gonad.

Kissing gourami fish is one of the swamp fish in South Kalimantan. Kissing gourami fish is also one of the swamp fish traded in markets in South Kalimantan. The taste of Kissing gourami fish meat is savory and can be used as a source of animal protein, where according to Kissing gourami fish itself (Dekawadi, 2019) Kissing gourami fish has a protein content of 16.70%.

Fish gonad maturation through feeding is usually done by adding feed ingredients that are generally added such as adding protein content to feed, oodev and adding vitamins to feed. The addition of vitamins to feed for gonadal maturation has also been carried out. One type of vitamin used is Vitamin E. The addition of Vitamin E for gonad maturation in Kissing gourami fish feed itself is still not widely done.

Vitamin E is an indispensable micronutrient and plays an important role in the growth, reproduction, and health of fish (Hunt *et. al*., 2004 *in* Pamungkas, 2013). The use of Vitamin E in increasing fish reproduction according to (Mustika, 2005) states that Vitamin E plays an important role as an antioxidant in maintaining the presence of fatty acids during gonadal development and accelerating the formation of reproductive hormones. Glutathione is an enzyme in the body that is an antioxidant. Research on the use of glutathione and Vitamin E in Kissing gourami fish is currently still rarely done.

The development of the fish gonad is closely related to the performance of the fish liver. Fish liver itself plays a role in the process of vitellogenesis. The process of vitellogenesis in the liver of fish will trigger the accumulation of fat in the liver of fish, according to (Simanjuntak, 2017) accumulation of fat in the liver of fish can heavier the work of the liver so that the physiological condition of fish decreases. The increase in the development of fish gonad can be seen by observing the gonad of fish. Observation of the gonad maturity level of Kissing gourami fish can be done by observing the histology of two organs, that is liver and gonads. Observation of histology is very important to know the activities outside of spawning related to the condition of the gonad of the fish be cared.

This research aims to determine the optimal dose of Vitamin E in commercial feed containing glutathione for the liver and gonad of Kissing gourami fish and to determine the histology of the liver and gonad of Kissing gourami fish feeded with commercial feed containing glutathione and Vitamin E.

MATERIALS AND METHODS OF RESEARCH

This research was conducted for ± 5 weeks. The research location at the Puja Kesuma fish pond, Sungai Sipai Irrigation, Martapura Sub-District, Banjar Regency, South Kalimantan Province.

Tools used: net cage, rope, wooden stake, basin, sprayer, water quality tool, stationery, sample bottle, treatment label, silage protection net, surgical instrument, digital scale, ruler, jar, styrofoam box, plastic clip, and sticky label.

Ingredients used: Kissing gourami fish, commercial feed branded Cheiljedang Galaxy superior catfish feed size 2, protein 35%, Vitamin E branded nature E 300, glutathione branded reduced L-gluthathione 300 mg, egg white, alcohol 70%, aquadest, and ice cubes.

The research procedures carried out included: stocking of test animals, feeding fish, observing water quality, taking samples of fish liver and gonad, observing histology and measuring egg diameter.

The distribution of test animals, namely Kissing gourami fish, in one treatment net cage was filled with 5 fish. Feeding ad satiation to the test fish was carried out 2 times a day, the test feed was given for ± 5 weeks. Water quality parameters observed were: dissolved oxygen, ammonia, temperature, and pH. Water quality observations were carried out at the Water Quality Laboratory Faculty of Fisheries and Marine Science. Sampling of the liver and gonad of Kissing gourami fish was carried out at the beginning and end of the research, liver and gonad samples were taken by dissecting the fish. Liver and gonad that have been weighed are stored for histological observation. Parameters measured included body weight, liver weight and gonad weight of fish. Histological observations of Kissing gourami fish were carried out in this research, namely observing the histology of the liver and gonad of Kissing gourami fish. Histological observations were carried out at the Banjarbaru Veterinary Center. Egg samples were measured using a microscope to get the diameter size of the eggs.

The experimental design used in this research was a completely randomized design (CRD):

- Treatment A = Commercial feed, Glutathione 10 mg/Kg;
- Treatment B = Commercial feed, Vitamin E 300 mg/Kg, Glutathione 10 mg/Kg;
- Treatment C = Commercial feed, Vitamin E 500 mg/Kg, Glutathione 10 mg/Kg;
- Treatment D = Commercial feed, Vitamin E 700 mg/Kg, Glutathione 10 mg/Kg.

Parameters observed in this research consisted of Hepatosomatic Index (HSI), Gonadosomatic Index (GSI), egg diameter, fecundity, liver histology, gonad histology, and water quality.

Quantitative data analysis in this research was carried out on the parameters of the Gonadosomatic Index (GSI), Hepatosomatic Index (HSI), egg diameter, and fecundity. The data obtained from the results of further research were tested for normality using the Liliefors test. Homogeneity test, using the Bartlett procedure. The normal and homogeneous data were analyzed for variance (ANOVA) to see the significance.

RESULTS AND DISCUSSION

The results of the Liliefors HSI Kissing gourami fish normality test obtained a L Max (0.129) < L Table 5% (0.242) the data is normally distributed. Bartlett homogeneity test obtained X^2 (0.645) <X^2 Table 5% (7.814), homogeneous data. Test (ANOVA) obtained F Count (2.987) < F Table 5% (4.066), so that H_0 = accepted, the addition of Vitamin E to commercial feed containing glutathione did not significantly affect the Hepatosomatic Index (HSI) of Kissing gourami fish.

Based on the orthogonal polynomial regression test of the Kissing gourami fish HSI parameter, it was found that the optimum value was 282 mg/Kg dose of Vitamin E.

The addition of Vitamin E also provides benefits for fish according to (Wahyudi, 2016) vitamin E also functions as a protective cell wall from materials such as mercury, mercury, 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.

The optimum dose of vitamin E on the hepatosomatic index in this research was obtained which was 282 mg/Kg of feed. The optimum dose of vitamin E in this feed is still in the dose range of this research. The optimum dose is relatively close to the results of the research (Muhlisin, et al., 2016) where the dose of vitamin E of 300 mg/Kg of feed is the optimum dose for fish IHS.

Table 1 – Average Results on Parameter

Parameters	Treatment			
	A	B	C	D
HSI	0.771 ±0.062[a]	0.925 ±0.093[a]	0.952 ±0.084[a]	0.735 ±0.110[a]
GSI	8.659 ±3.680[a]	9.890 ±1.755[a]	9.484 ±1.146[a]	9.148 ±3.149[a]
Egg Diameter	1302.5 ±6.8[a]	1325.6 ±52.1[a]	1241.1 ±74.0[a]	1320.6 ±14.9[a]
Fecundity	21907.6 ±10004.9[a]	19056.0 ±1390.7[a]	21632.0 ±3442.9[a]	18197.3 ±7511.2[a]

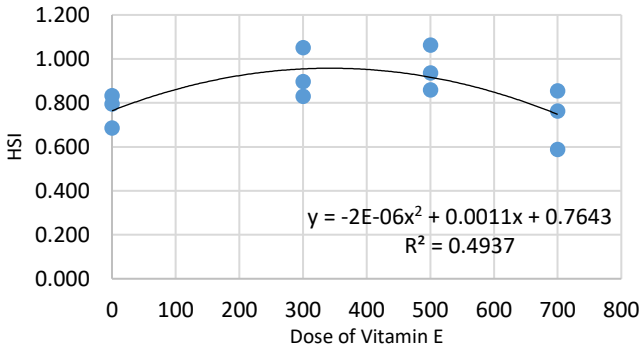

Figure 1 – Point distribution and HSI curve of Kissing gourami fish

The results of the Liliefors GSI Normality Test of Kissing gourami fish obtained a L Max (0.159) < L Table 5% (0.242) data is normally distributed. Bartlett homogeneity test obtained X^2 (2.931) < X^2 Table 5% (7.814), homogeneous data. Test (ANOVA) obtained F Count (0.078) < F Table 5% (4.066), so H0 = accepted, the addition of Vitamin E to commercial feed containing glutathione had no significant effect on the Gonadosomatic Index (GSI) of Kissing gourami fish.

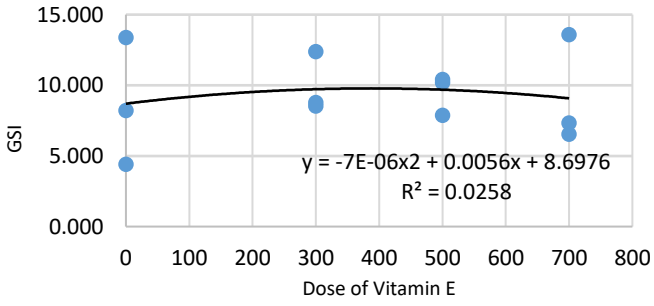

Figure 3 – Distribution point and GSI curve of Kissing gourami fish

Based on the orthogonal polynomial regression test on the GSI parameters of Kissing gourami fish, the optimum value was 397.143 dose of Vitamin E.

Based on the overall results of the treatment on the gonadosomatic Index (GSI) parameter obtained during the reseach, the GSI values ranged from 4.399 to 13,578. While the results of the research (Gunarsa, 2017) the GSI values obtained ranged from 5.71-13.26. The large GSI value obtained during the research was due to the condition of the Kissing gourami fish which was getting closer to maturity, the size and weight of the fish gonad that were approaching maturity would increase and the belly of the Kissing gourami fish would look relatively fuller. Based on the results of the ANOVA test of GSI data, it was found that the addition of Vitamin E to commercial feed containing glutathione had no significant effect on the Gonadosomatic Index (GSI) of Kissing gourami fish.

Fat found in the liver is also found in the gonad, this is characterized by the condition of the gonad when touched, slippery like oily (fatty), fat in the gonad itself is needed in the vitellogenesis process to avoid the loss of fat, vitamin E is needed. Addition of vitamin E to feed can increase the GSI value due to the function of vitamin E, namely as an antioxidant. Vitamin E can prevent the oxidation of fats which are needed in the process of vitellogenesis or the manufacture of egg yolks. The more fat, the more vitellogenin produced by the liver. Increasing the amount of vitellogenin in oocytes can increase gonadal weight so that the GSI value will also increase. The greater the GSI percentage value, the higher the level of egg maturity (Erly, 2020). The optimum dose of vitamin E on the gonadosomatic index of this research was obtained, which was 397.143 mg/Kg of feed. The optimum dose of Vitamin E of 397.143 mg/Kg of this feed is still in the dose range of this research. The optimum dose is relatively high from the results of the research (Darwisito, 2008) the optimum dose of vitamin E on the fish gonadosomatic index was obtained, which was 150 mg/Kg of feed.

The results of the Liliefors Normality Test for egg diameter of Kissing gourami fish obtained a L Max (0.179) < L Table 5% (0.242), the data is normally distributed. Bartlett homogeneity test obtained X^2 (4,862) <X^2 Table 5% (7,814), homogeneous data. Test (ANOVA) obtained F Count (2.351) < F Table 5% (4.066), so H_0 = accepted, the addition of Vitamin E to commercial feed containing glutathione did not significantly affect the egg diameter of Kissing gourami fish.

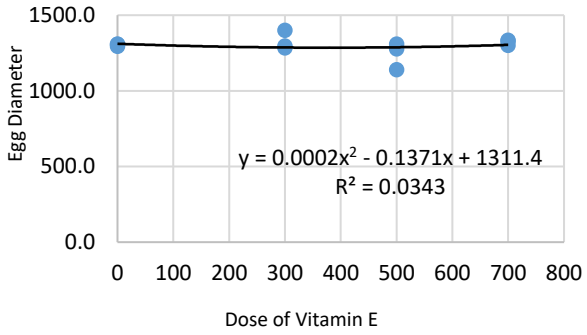

Figure 2 – Point Distribution and Egg Diameter Curve of Kissing gourami fish

Based on the orthogonal polynomial regression test on the egg diameter parameter of Kissing gourami fish, the optimum value was 342.750 doses of Vitamin E.

Based on the overall results of the treatment on the size parameters of the Kissing gourami fish egg diameter during the study, the results obtained ranged from 1138.2 m to 1398.8 m. While the diameter size of the Kissing gourami fish eggs obtained by the research (Susilo, 2019) ranged from 820-900 m. The large size of the egg diameter obtained during the research was due to the size of the fish used at the time of the research which was relatively larger than that used in previous research.

The optimum dose of vitamin E in the egg diameter of the Kissing gourami fish in this research was obtained which was 342.750 mg/Kg of feed. The optimum dose of Vitamin E of 342.750 mg/Kg of this feed is still in the dose range of this research. The optimum dose is relatively close to the results of the research (Tarigan, 2017) where the best dose of Vitamin E of 375 mg/Kg of feed has an effect on the egg diameter of fish.

The results of the Liliefors normality test for the fecundity of Kissing gourami fish obtained a L Max (0.195) < L Table 5% (0.242), the data is normally distributed. Bartlett homogeneity test obtained X^2 (6.467) <X^2 Table 5% (7.814), homogeneous data. Test (ANOVA) obtained F Count (0.161) < F Table 5% (4.066), so H_0 = accepted, the addition of Vitamin E to commercial feed containing glutathione did not significantly affect the fecundity of Kissing gourami Fish.

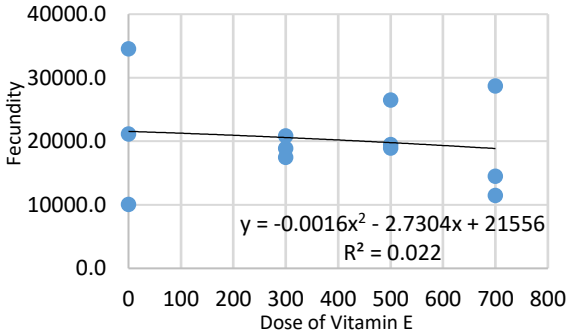

Figure 3 – Distribution Point and Fecundity Curve of Kissing gourami fish

Based on the orthogonal polynomial regression test on the fecundity parameter of Kissing gourami fish, the optimum value was 853,250 dose of Vitamin E.

Based on the overall results of the treatment on the fecundity parameter or the number of Kissing gourami fish eggs obtained during the research, it was found that the fecundity ranged from 10064 to 34533.3 eggs. Meanwhile, the results of the fecundity of research Kissing gourami fish (Ningtias, 2017) obtained ranged from 3079-26046. The amount of fecundity or the number of eggs produced during the research was due to the size of the fish used at the time of the study being relatively larger than those used in previous studies, the large size of the fish will make the number of eggs produced relatively more. The optimum dose of vitamin E in the fecundity of this study was obtained in the amount of 853.250 mg/Kg of feed. The optimum dose of Vitamin E of 853.250 mg/Kg of this feed is outside the dosage range of this research. The optimum dose is relatively high from the results of research (Tarigan, 2017) where the best dose of Vitamin E of 375 mg/Kg of feed has an effect on fish fecundity.

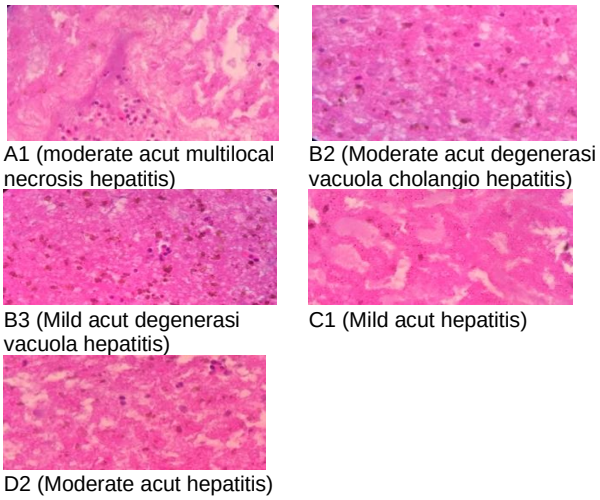

Figure 4 – Histology liver of Kissing gourami fish

In the liver of Kissing gourami fish, symptoms of inflammation occur, in an inflamed liver there is cell damage (necrosis). Liver cell necrosis is a process of cell death after cell injury. Cell death is characterized by three changes in the cell nucleus, namely 1) pyknosis in

which the cell nucleus looks round, dark and smaller than the normal cell nucleus, 2) karyorexis with the cell nucleus splitting into several parts, 3) karyolysis in which the chromatin of the cell nucleus disappears and leaves holes in cells (Thompson, 1984 in Auliyah, 2016).

Necrosis itself is divided into several grades based on certain assessment criteria: Necrosis in Hepatocyte Cells according to (Schafer et al., 2018): Grade 0: within normal limits, grade 1 (minimum): approx. <5% of liver (hepatocytes) necrosis, grade 2 (mild): about 5% to 20% of liver (hepatocytes) are necrotic, grade 3 (moderate): about 20% to 40% of liver (hepatocytes) are necrotic, and grade 4 (marked)/severe): generally >50% of the liver (hepatocytes) are necrotic. Some treatments were found to have multifocal and focal necrosis. Multifocal necrosis is necrosis that occurs in groups, can occur due to bacterial infection. Necrosis is damage to liver cells. Multifocal necrosis is necrosis of many cells in one place. Focal liver cell necrosis is necrosis that occurs randomly in one cell or a small group of cells throughout the liver lobule (Oei, 2013; Firdauzi, 2018). Some treatments found vacuolar degeneration or swelling of the vacuoles and cholangitis was also found. Degeneration is the accumulation of fluid in the liver with the characteristics of swollen liver cells and the cytoplasm looks cloudy. Cholangitis is the term used for inflammation of the walls of the bile ducts, almost always caused by infection of the lumen. This situation can come from any lesion that blocks the bile duct (Auliyah, 2016).

In treatment A without being given Vitamin E (0 mg/Kg) there was moderate acute multifocal necrosis. In treatment B with a dose of Vitamin E (300 mg/Kg) there was moderate acute degeneration of the vacuole cholangio hepatitis. In the B3 treatment with a dose of Vitamin E (300 mg/Kg) there was mild acute vacuole degeneration of hepatitis. In treatment C with a dose of Vitamin E (500 mg/Kg) there was mild acute hepatitis. Meanwhile in treatment D with a dose of Vitamin E (700 mg/Kg) there was moderate acute hepatitis.

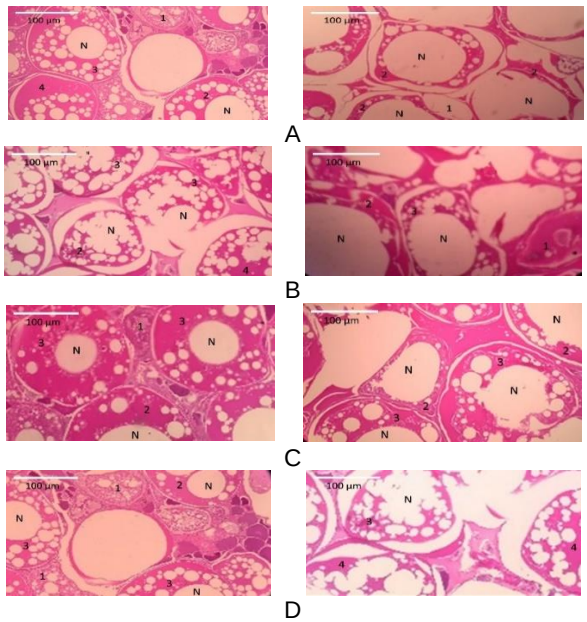

Figure 5 – Histology gonad of Kissing gourami fish: Histology of gonads of Kissing gourami fish (A: 0 mg/Kg Vitamin E, B: 300 mg/Kg Vitamin E, C: 500 mg/Kg Vitamin E, D: 700 mg/Kg Vitamin E); N: Nucleus, 1: oocyte stage I, 2: oocyte stage II: 3: early vitellogenesis, 4: yolk begins to fill the ooplasm, Arrow: yolk granules.

The gonad histology of Kissing gourami fish in the research that has been carried out found that the diameter of the eggs has been formed and is still not uniform and has entered the maturing stage. This can be seen from the discovery of the nucleus of the egg (nucleus) so that the egg has not fused.

In treatment A with a dose of Vitamin E (0 mg/Kg), stage I oocytes, stage II oocytes, stage III oocytes (early vitellogenesis) were found and up to stage IV oocytes or egg yolks that began to fill the ooplasm, in other A treatments oocytes were found. stage I, and predominately stage II oocytes. Furthermore, in treatment B with a dose of Vitamin E (300 mg/Kg), stage II oocytes and stage IV oocytes or egg yolks were still found which began to fill the ooplasm with a predominance of stage III oocytes (early vitellogenesis), in other B treatments, stage I oocytes were found, stage II oocytes and stage III oocytes (early vitellogenesis). Meanwhile, in treatment C with a dose of Vitamin E (500 mg/Kg), stage I and stage II oocytes were still found, dominated by stage III oocytes (early vitellogenesis), in other C treatments, stage II oocytes and stage III oocytes (early vitellogenesis) were found. vitellogenesis). Then the situation in treatment D with a dose of Vitamin E (700 mg/Kg), stage II oocytes were still found, dominated by stage I oocytes and stage IV oocytes or egg yolks that began to fill the ooplasm, in other D treatments, stage III oocytes were found (early vitellogenesis), and predominately stage IV or yolk oocytes that begin to fill the ooplasm.

Based on the histological observations of the gonad obtained, it was found that the oocyte pattern of the Kissing gourami fish was diverse; the diverse oocyte pattern indicated that the Kissing gourami fish was a partial spawner. This is in accordance with the results of research (Lisna, 2016) that Kissing gourami fish are fish that have a partial spawner that release their eggs gradually.

Table 2 – Water quality

No.	Parameters	Value		Literature
		Early	End	
1.	Temperature (°C)	29	30	25-30 (Yurisman, 2009 *in* Azhari, 2020).
2.	pH	6.8	6.6	6,5–7 (Susilo, 2019).
3.	Dissolved Oxygen (mg/L)	3,35	3,52	> 3 (Arifin, 2017).
4.	Ammonia (mg/L)	0,01	0,26	<1 (Santi, 2021).

CONCLUSION

The administration of vitamin E in commercial feed containing glutathione did not affect the HSI and GSI of Kissing gourami fish, the highest yield of HSI of Kissing gourami fish was treated with C (500 mg dose of Vitamin E) of 0.952 and the highest yield of Kissing gourami GSI was treated with B (300 mg dose of Vitamin E) of 9.890. The optimal dose for HSI Kissing gourami fish is 282 mg/Kg of Vitamin E and the optimal dose for GSI of Kissing gourami fish is 342.750 mg/Kg of Vitamin E.

Liver histology, in the end it was found that the liver of the fish had liver cell damage. Meanwhile, in gonad histology, it was found that the oocyte pattern of Kissing gourami fish phase I-to phase IV oocytes or egg yolks that begin to fill the ooplasm showed that the Kissing gourami fish were partially spawner fish.

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