Effect of honey substitution in feed on gonad development of female Tilapia (*Oreochromis niloticus* Linnaeus, 1758)

Firdus Firdus[1,*], Nur Azizah[1], Widya Sari[1], Cut Nanda Devira[1], Alia Rizki[1], Dahlan Dahlan[1], Agung Setia Batubara[2]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala, Banda Aceh, Indonesia

[2]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Medan, Sumatera Utara, Indonesia

*Email: firdus@unsyiah.ac.id

Received 4 February 2022; Received in revised from 14 May 2022; Accepted 1 June 2022

Abstract *This research was conducted to determine the effects of feeding with several honey contents to the gonad maturation of female Tilapia (Oreochromis niloticus). This study used a completely randomized design (CRD). The research was divided into four treatments and five replications. The treatments consisted of feeding honey with a dose of 0 ml/kg feed (P0=control), 100 ml/kg feed (P1), 200 ml/kg feed (P2), and 400 ml/kg feed (P3). The parameters observed in this research were the gonad development stage (GDS), gonadosomatic index (GSI), and fecundity. The results showed that the treatment had a significant effect (P<0.05) on GDS, GSI, and fecundity of the female Tilapia. Feeding with a dose of 400 ml honey/kg feed gives the best value for GDS, GSI, and fecundity.* [EFFECT OF HONEY SUBSTITUTION IN FEED ON GONAD DEVELOPMENT OF FEMALE TILAPIA (*OREOCHROMIS NILOTICUS* LINNAEUS, 1758)] ***(J. Math. Nat. Sci., 2(1): 1 - 6, 2022)***

***Keywords*:** *Fecundity, Gonad, Feed, Index*

Introduction

Tilapia (*Oreochromis niloticus*) is one type of fish that has high economic value, where the need for seeds and fish consumption from year to year tends to increase along with the expansion of aquaculture. However, there are still some obstacles that have not been met until now, namely the low quality of seeds, unavailability of seeds at any time on an ongoing basis, low egg fertilization and egg hatching rates. One of the causes of the decline in the quality of eggs and larvae is thought to be due to the low quality of the parent feed given. Parent feed which is generally used in tilapia hatchery is commercial feed for rearing and not specially made feed for hatchery purposes, so that the results obtained are not optimal in terms of both quality and quantity of seeds.

One way to obtain optimal fish hatchery results is to improve reproductive performance, where reproduction can be improved, among others, by improving the nutritional quality of broodstock feed. Value fish that have easy breeding properties result in decreased growth of 10-20% per generation which is characterized by smaller body size, slow growth and fast gonad maturity at small sizes (Yuliati et al., 2003). The small size of the gonads makes the mother tilapia will produce a few seeds with a small size as well. Therefore, the handling of broodstock needs attention, this is related to the success of the gonad maturation process.

Many efforts have been made to stimulate gonadal maturity, including environmental manipulation and hormone administration. However, this approach is expensive and can leave unfavorable residues when consumed, thereby reducing food safety (Effendi, 1997). One alternative that can be done to produce freshwater tilapia which has superior gonad quality naturally is by giving honey supplements added to the feed. Honey contains simple sugars (monosaccharides) such as fructose and glucose which are needed by spermatozoa. Based on data from the United States Department of Agriculture (USDA), honey contains 38% fructose; 31% glucose; 17.1% water; 7.2% maltose; 4.2% trisaccharides and some polysaccharides, 1.5%

sucrose, 0.5% minerals, vitamins and enzymes (Hidayaturrahmah, 2010).

Utilization of honey has been widely used in research in the field of fisheries. These studies include the addition of honey to tilapia sperm dilution material (Yunus et al., 2015). Soaking tilapia larvae in honey solution (Agus et al., 2007). The use of honey as a diluent for storage of catfish spermatozoa (*Pangasius pangasius*) (Dea, 2005). But so far there is still not much information on the use of honey as an ingredient added to feed. In connection with this, the purpose of this study is to make honey as a feed ingredient that can have a good effect on the development of fish gonads.

The addition of honey in the feed is to help accelerate the process of gonad maturation so that the reproductive process can be accelerated. Good food will support the work of the body's organs so that they can work better. Including hormones and the endocrine system. The endocrine system is very helpful in the reproductive process, namely by regulating the function of the reproductive organs.

Research Methods

Research time and site. This research was conducted from December 2016 to March 2017. The research was carried out at the Hatchery II Center for Brackish Water Aquaculture (BPBAP) Ujong Batee, Aceh Besar District, Indonesia.

Tools and materials. The tools used are plastic containers (25L volume), buckets, aeration, scoops, scoops, scissors, digital scales, spatulas, cameras, caliper, calipers, feed printing machines, trays, and stationery. The materials used were honey, female tilapia, gloves, masks, fish feed and 0.9% NaCl solution.

Method. This study used a completely randomized design (CRD), the test animals were divided into four treatments and five replications. The treatments consisted of feeding honey with a dose of 0 ml/kg feed (P0=control), 100 ml/kg feed (P1), 200 ml/kg feed (P2), and 400 ml/kg feed (P3). The total number of test animals used was 20 samples. The basis for determining the dose refers to the research conducted by Syaifuddin (2004).

Fish preparation and treatment. This study used female tilapia that had been cultivated at BPBAP Ujung Batee, Aceh Besar. The number of fish used is 20 tails. This tilapia was initially kept for a week in a plastic container and then treated in the form of feeding with honey added at 08.00 WIB and 17.00 WIB for 21 days (Mukti, 2002). During maintenance, the broodstock were given feed that had been mixed with bee honey according to treatment (0, 100, 200, and 400 ml/kg of feed) with a protein content of 30% as much as 3% of body weight.

Observation of the shape, color and content of the gonads. Observation of the gonads was carried out by dissecting the fish stomach using surgical instruments. Surgery starts from the anal to the base of the head horizontally. The skin and flesh of the fish belly are opened and the digestive tract is set aside to make the gonads clearer. The shape and color of the gonads are seen, then the tilapia gonads are split open to see the inside and the results are recorded.

Measurement of length and weight of gonad. Tilapia gonads were then measured using a caliper and weighed using an analytical balance with an accuracy of 0.01 g, then the results were recorded. Gonads were weighed to obtain the gonadosomatic index (GSI). According to Effendi (2002), the GSI value is calculated using the formula: GSI = WG/WB×100, Where GSI = Gonadosomatic index (%), WG = Weight of gonad (g), WB = Weight of body (g).

Table 1. Determination of gonad development stage (GDS) score

Score	Characteristics of gonads and eggs
0	Ovaries are oval, reddish in color with capillaries. Fills approximately half of the lower abdominal space. Eggs are visible to the eye. There are still many eggs with morphology and color like white powder.
1	Ovaries are oval, yellowish in color with capillaries. Fills approximately of the lower abdominal space. Eggs can be seen by the eye, eggs that are like white powder are decreasing and the majority of eggs are yellow.
2	Ovaries are oval, yellowish in color with capillaries. Fills approximately of the lower abdominal space. Eggs can be seen by the eye, very few eggs are like white powder.
3	The ovaries are full (plump), the ovaries are yellowish, filling more than of the lower space, the eggs can be seen by the eye, the eggs are round, some of them are clear and ripe.

***Gonad development stage* (*GDS*)**. GDS observations of tilapia were based on the size, color, and weight of the gonads and the ratio of the gonads and the abdominal cavity. Observations also include the size, color and shape of eggs based on Bagenal and Braum (1968). In order for the data to be analyzed quantitatively, further modifications were made based on changes in the condition of the gonads and eggs using a scoring system (Table 1).

Fecundity. Determination of fecundity in this study was using the manual method. This method is the best and most accurate way. How to count eggs one by one from the gonads. Fecundity were performed immediately after gonadal measurement.

Data analysis. Data obtained from observations of GDS, GSI, and fecundity, then analyzed with one-way variance and continued with Duncan Multiple Range Test.

Results

Tilapia that were given the test feed treatment with various doses of adding honey turned out to have different gonadal maturity. This indicates that the different doses of honey in the test feed affected the gonad development of tilapia. The GSI value of tilapia ranged from 1.45% - 5.16%. The results of analysis of variance on the GSI value of tilapia broodstock at various doses of adding honey in the feed showed an effect ($P<0.05$). After being continued with Duncan's multiple-distance test, the results were significantly different (Table 2).

The results of observations on the gonad development stage (GDS) based on gonadal morphology showed that the gonads of tilapia (P0) had a round shape with reddish to yellowish ovary color with various egg morphology conditions. The results of observations on gonadal morphology showed that the application of honey had a significant effect on the GDS value. The results of statistical analysis showed an increase in the average GDS value in line with the increase in the dose of honey (Table 2).

Tilapia which was given the test feed treatment with different doses of honey, in fact experienced an increase in fecundity in line with the increase in the dose of honey given. The results of analysis of variance showed that the dose of honey treatment had an effect on the mean fecundity, the higher the dose of honey, the higher the number of eggs produced. Duncan test results can be seen in Table 2.

Table 1. Statistical analysis results of gonadosomatic index (GSI) and gonad development stage (GDS) of female tilapia

Variables	Treatments			
	P0	P1	P2	P3
GSI	$1.92^{a} \pm 0.37$	$2.85^{b} \pm 0.41$	$3.65^{c} \pm 0.37$	$4.27^{d} \pm 0.63$
GDS	$0.9^{a} \pm 0.28$	$1.30^{b} \pm 0.16$	$1.64^{c} \pm 0.13$	$1.75^{c} \pm 0.16$
Fecundity	$144.60^{a} \pm 25.25$	$235.20^{b} \pm 46.72$	$313.80^{c} \pm 50.10$	$336.00^{c} \pm 28.77$

Where: Numbers followed by different superscripts show significant differences between treatments. The treatments consisted of feeding honey with a dose of 0 ml/kg feed (P0=control), 100 ml/kg feed (P1), 200 ml/kg feed (P2), and 400 ml/kg feed (P3).

Observation of ovarian morphology showed differences between control fish gonads compared to fish gonads with the addition of honey in the feed. After 21 days, the gonadal morphology of P1, P2, and P3 treated fish was more developed than the control, where the control growth was slower based on oocyte maturity and smaller in size (Figure 1).

Discussion

Table 1 shows that the mean GSI values of tilapia were significantly different between treatments ($P<0.05$). The mean value of GSI increased with the addition of the dose of honey in the feed. The addition of honey increased the gonad weight of the tilapia which led to an increase in the GSI value. The gonads of oviparous animals such as fish enlarge in line with the increase in the size of the egg they contain. The egg cell will increase in size in line with the synthesis of yolk in the vitellogenesis process. The content of honey in the feed is thought to increase the mechanism of vitellogenesis in tilapia. Tyler (1991) stated that the increase in GSI values could be caused by oocyte development. Oocytes develop in parallel with vitellogenesis. Vitelogenin is the egg yolk which is the main component of the growing oocyte. Yaron (1995) stated that during the vitolegenesis process, egg yolk granules increase in number and size so that the oocyte volume increases and will eventually cause the GSI value to increase.

The mineral content in honey such as potassium, calcium, magnesium, sodium and phosphate is thought to help increase the gonad maturity of tilapia. According to Marhiyanto (1999) that in every 100 g of honey contained 205-1676 ppm potassium, 49-51 ppm calcium, 19-35

ppm magnesium and 18 ppm sodium. Syaifudin (2004) argues that the high potassium contained in the honey supplement given affects the change in cholesterol contained in fish tissue to pregnenolone. Pregnenolone is a source of biosynthesis of steroid hormones by the adrenal glands, these steroids affect the formation of gonadal hormones and the development of gamete cells.

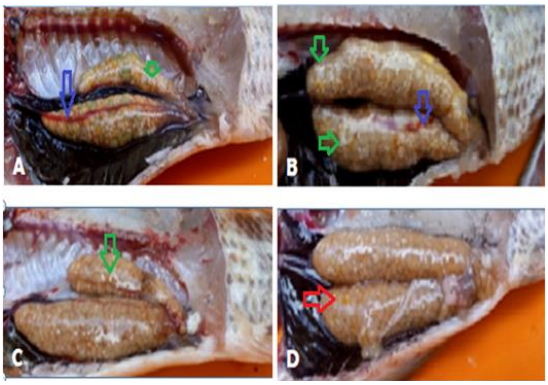

Figure 1. Gonad morphology of female tilapia, where (a) Gonads treated with 0 ml honey/kg feed, (b) Gonads treated with 100 ml honey/kg feed, (c) Gonads treated with 200 ml honey/kg feed, (d) Gonads treated with 400 ml honey/kg feed. Blue arrows = capillaries, green arrows = white powder, red arrows = mature eggs.

Furthermore, the mineral content of honey is thought to help regulate reproductive hormones that play a role in the vitellogenesis process. According to Mommsen and Walsh (1988), the mineral content in food can affect the activity of hypothalamic neuroendocrine cells. GnRH secreted into the blood will stimulate the pituitary to secrete gonadotropin hormones. The gonadotropin hormone then triggers the follicular cells to produce the hormone estrogen. The hormone estrogen in blood plasma will induce liver cells to synthesize vitellogenin.

The increase in gonadal weight was thought to be due to an increase in the secretion of vitellogenin (egg yolk) induced by the honey content in the form of calcium, potassium and phosphate. According to Björnsson and Haux (1985), vitellogenin is a protein that binds to calcium, potassium and inorganic phosphate. Plasma levels of calcium, potassium and inorganic phosphate physiologically regulate vitellogenin synthesis. The beta-carotene content of honey can affect the breeding process because it stimulates the fertilization process. Parwata et al. (2010), stated that the beta carotene content in honey reached 1.9687 mg/100g to 3.6327 mg/100 g.

Gonadal development occurs when there is an excess of energy for maintenance of the body, while nutritional deficiencies can cause eggs to experience atresia. According to Watanabe et al. (1988) and Mokoginta (1992) of several factors that can affect gonad maturation, feed quality has a very close relationship with the quality of the eggs produced. The effect of feed on gonadal development was reported by Lovell (1988) that the gonad maturity of Channel catfish is closely related to the balance of the nutritional composition of the feed which is needed for gonadal development. Each species of fish requires good nutrition, namely protein, fat, carbohydrates, vitamins, and minerals as well as energy for life activities. According to NRC (1983), energy is needed by fish for metabolic processes, body care, physical activity, growth and reproduction. Edimarwan (2006) said that nutrient deficiency, especially amino acids, vitamins, and minerals can cause delayed egg development and ultimately failure of ovulation and spawning.

The value of fecundity of a fish species is influenced by several factors including feed, fish size, egg diameter, and environmental factors. In addition, fecundity is a subject that can adapt to various conditions, especially the response to feed (Effendie, 2002). Honey added to the feed will provide a supply of minerals and carbohydrates for the production of tilapia vertebrate oocytes. Vitelogenin is the main component of fish oocytes. The supply of vitellogenin comes from outside the ovary. Vitelogenin is derived from the vertebrate liver which is carried by the blood to the oocyte. Vitellogenin is classified as a phospholipoglycoprotein molecule composed of a protein functional group bound to a lipid, some carbohydrates, and a phosphate group. In addition, vitellogenin also has strong ion-binding properties and thus can serve as the main mineral supply for oocytes (Heffner and Danny, 2008).

Fish fecundity is influenced by hormonal activity, where the clutch rhythm is controlled by Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) through a positive feedback mechanism between the ovaries and the pituitary-hypothalamus (Patino and Sullivan, 2003). Systemic inclusion of honey can increase the release of hormones that play a role in egg production, thereby increasing the fecundity of tilapia. According to Mommsen and Walsh (1988), eating activity will stimulate the hypothalamus to

secrete Gonadotropin Releasing Hormone (GnRH). Mineral content in food can affect the activity of hypothalamic neuroendocrine cells. GnRH secreted into the blood will stimulate the pituitary to secrete gonadotropin hormones. The gonadotropin hormone then triggers the follicular cells to produce the hormone estrogen. The hormone estrogen in blood plasma will induce liver cells to synthesize vitellogenin. Sundararaj (1981) During vitellogenesis, the hormone estrogen produced by the ovaries is released into the blood, then stimulates the liver to synthesize vitellogenin. After being synthesized, vitellogenin is released into the bloodstream and then selectively absorbed by the oocyte, as a result, the egg cell will grow bigger and ready to be fertilized.

Conclusion

Based on the results of the study, it was concluded that increasing the dose of honey in tilapia broodstock feed had a significant effect on reproductive performance, especially on gonadal development and egg quality. Feed containing honey 400 ml/kg of feed gave the best effect on improving the quality of tilapia eggs, which was indicated by the yellow color of the eggs.

References

Arsetyo, A., Rahardhianto, R., Abdulgani, N., Trisyani, N. (2012). Pengaruh konsentrasi larutan madu dalam NaCl fisiologis terhadap viabilitas dan motilitas spermatozoa ikan patin (*Pangasius pangasius*) selama Masa Penyimpanan. *J. Sains Seni ITS*, 1: 58-63

Ayer, Y., Mudeng, J., Sinjal, H. (2015). Daya tetas telur dan sintasan larva dari hasil penambahan madu pada bahan pengencer sperma ikan nila (*Oreochromis niloticus*). *J. Bud. Per.*, 3: 149 – 153

Bagenal, T.B., Braum, E. (1968). Eggs and early life history, in W.E. Ricker ed. Methods for assesments of fish production in fresh water

Bjornsson, B.T., Haux, C.H. (1995) Distribution of calcium, magnesium, and inorganic phosphate in plasma of estradiol -17β treated rainbow trout. *J. Comp. Physiol. B*, 155: 347-352

Edimarwan, E. (2006). Pengaruh tepung ikan lokal dalam pakan induk terhadap pematangan gonad dan kualitas telur ikan baung (*Mystus nemurus*). Tesis Fakultas Perikanan. Institut Pertanian Bogor. Indonesia

Heffner, L.J., Danny, J.S. (2008). At a glance sistem reproduksi. Edisi kedua. Penerbit Erlangga, Jakarta

Hidayaturrahmah, H. (2010). Waktu motilitas dan viabilitas spermatozoa ikan mas (*Cyprinus carpio*) pada beberapa konsentrasi larutan fruktosa. *Bioscientiae*, 4(1): 11

Effendie, M.I. (2002). Biologi perikanan. Yayasan Pustaka Nusatama, Yogyakarta

Irianto, A., Watung, J., Kalesaran, O., Monijung, R. (2014). Penambahan madu dalam pengenceran sperma untuk meningkatkan motilitas spermatozoa, fertilisasi dan daya tetas telur ikan lele (*Clarias* sp). J. Bud. Per., 3: 27-34

Lovell, R.T. (1988). Increasing omega-3 fatty acids in farmed catfish. *Aquaculture*, 141: 49-55

Marhiyanto, B. (1999). Peluang bisnis beternak lebah. Gita Media Press. Surabaya

Mokoginta, I. (1992). Essential fatty acid requirements of catfish (*Clarias batracus* Linn.) for broodstock development. Disertasi. Fakultas Pascasarjana, Institut Pertanian Bogor

Mommsen, T.P., Walsh, P.J. (1988). Vitellogenin and oocyte assembly. P: 525-567. In: W.S. Hoar, D.J., Randall dan E.M. Donaldson, (Eds.). Fish Physiology. Vol. ke-11, part A. Reproduction. New York: Academic Press

Mukti, A.T., Rustidja, R. (2002). Peningkatan kualitas benih ikan gurami (*Osphronemus gourami*) dengan metode monosex jantan. *J. Med. Ked. Hew.*, 18: 104-107

NRC (National Research Council). (1983). Nutrient requirements of warm water fishes and shellfishes. National Academy of Science Press, Washington D.C

Parwata, A., Ratnayani, K., Ana, L. (2010). Aktivitas antiradikal bebas serta kadar beta karoten pada madu randu (*Ceiba pentandra*) dan madu kelengkeng (*Nephelium longata* L.). J. Kim., 4: 54-62

Patino, R., Sullivan, C.V. (2003). Ovarian follicle growth, maturation, and ovulation in Teleost fish. *Fish Physiol*. *Biochem*., 26: 57-70

Sundararaj, B.I. (1981). Reproductive physiology of Teleost fishes: A review of present knowledge and needs for future research. Aquaculture Development and Coordination Programme ADCP/REP/81/16. FAO Rome. Italy

Syaifuddin, A. (2004). Pengaruh pemberian suplement madu pada larva ikan nila GIFT (Oreochromis niloticus) terhadap rasio jenis kelamin. Thesis. Universitas Brawijaya, Malang

Tyler, C. (1991). Vitellogenesis in salmonid. P: 295-299. In: A.P. Scott, J.P. Sumpter, D.E. Kinne and M.S. Rolfe (Eds.). Proceeding of the Fourth International Symposium on the Reproductive Physiology of Fish. Norwich

Watanabe, T. (1988). Fish nutrition mariculture Jica texbook the general aquaculture course. Departement of Aquatic Biosiences Tokyo University of Fisheries, Japan

Wiryanta, W., Wahyu, W. (2010). Budidaya dan bisnis ikan nila. Agromedia Pustaka, Jakarta

Yaron, Z. (1995). Endocrine control of gametogenesis and spawning induction in the carp. *Aquaculture*, 129: 49-73

Yuliati, P., Kardarini, T., Rusmaedi, R., Subandiah, S. (2003). pengaruh padat penebaran terhadap pertumbuhan dan sintasandederan ikan nila gift (*Oreochromis niloticus*) di kolam. *J. Iktiol. Ind.*, 3: 63–66