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Individual Growth and Gonad Development Of Female Tilapia (*Oreochromis* Sp.) With Addition of Velvet Bean (*Mucuna Pruriens*) Seed Extract

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ABSTRACT

Tilapia is one of the fish that is widely cultivated in Indonesia. The obstacle to tilapia cultivation is high reproduction, which inhibits growth. An alternative to increase growth is by inhibiting gonad growth, so that feed energy is focused on somatic development. The aim of this research was to analyse the effect of velvet bean extract concentration on individual growth and gonad development of female tilapia fish and to determine the optimum concentration of velvet bean extract to increase growth and reduce reproductive activity in female tilapia fish. This study used a Completely Randomized Design (CRD) with the addition of various velvet bean extracts and compared with the control (without velvet bean extract). The use of velvet bean extract had a significant effect ($p<0.05$) on growth parameters such as length, weight, fecundity and egg diameter. The best treatment was found in treatment 3 (velvet bean extract 5 $ml.kg^{-1}$) which resulted in growth in individual length of 16.1 cm, individual weight of 89.43 g, fecundity of 2206 and egg diameter of 1.11 mm. In addition, the dose in this treatment may reduce the reproductive activity of tilapia.
Keywords: gonad; growth; tilapia; velvet beans

INTRODUCTION

Tilapia is a freshwater commodity that is widely cultivated in Indonesia. The production of Nile tilapia in Indonesia has increased since 2011, but in 2020 the aquaculture production is only 364,747.10 tons from 1,337,831.69 tons in 2019 (Marine and Fishery Ministry, 2021). Problems occur because, in the cultivation process, there are various obstacles including feed convention rate and high reproductive activity (Firdaus et al., 2020). Tilapia gonads mature for the first time at a size of 18 - 20 cm between 2 - 3 months of age (Chapman, 2012; Sapphire, 2018). Rapidly maturing gonads result in a reduction of body growth and population heterogeneity (Omitoyin et al., 2013). The slow body growth is because the energy that should be absorbed for somatic growth is used for gonadal development (Kapinga et al., 2018). The most widely used technique for increasing the production of tilapia aquaculture is using male monosex which is produced using 17a-methyltestosterone (MT) (Carman et al., 2008). However, the obstacles to monosex production are low availability and high prices. Furthermore, the usage of MT has been banned in Indonesia since 2014 based on the Decree of the Minister of Maritime Affairs and

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Fisheries No. KEP52/MEN/2014. It makes alternative materials needed to inhibit gonad growth and increase tilapia growth. Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) are hormones that regulate gonadal development. The hypothalamus controls the synthesis of FSH and LH through the synthesis of gonadotropin-releasing hormone (Hayati et al., 2019). Gonadotropin synthesis is regulated by the regulation of the hormone dopamine. Dopamine receptors will stimulate growth hormone (GH) and inhibit the synthesis of gonadotropin-releasing hormone in fish. Dopamine is a neurotransmitter that plays a role in inhibiting the synthesis of FSH and LH for gonadal maturation (Jiang et al., 2016; Bastiar et al., 2017). L-Dopa is a precursor of dopamine (Stansley and Yamamoto, 2013). Velvet bean seed contains 0.58 - 6.42% L-dopa (Pulikkalpura et al., 2015). Therefore, velvet bean seed can be a natural source of dopamine.

Several velvet bean seed studies have been carried out, such as the addition of velvet bean seed flour to African catfish (*Clarias Gariepinus*) which can increase the specific growth rate and feed efficiency at a dose of 5% of the feed ingredients (Aderolu and Akpabio, 2009). The addition of velvet bean seed extract increased the specific growth rate and efficiency level of *Rasbora Argyrotaenia* fish feed at a dose of 1 $ml.kg^{-1}$ of feed (Husnacahya, 2022). The addition of velvet bean seed extract also inhibits the reproduction time of *Moina macrocopa* with a concentration of 0.01% (Timur, 2021). Increased somatic growth occurs because dopamine has a positive effect on Growth Hormone (GH) production and a negative effect on FSH and LH production (Setyawan et al., 2014).

Based on these data, this study aims to analyse the effect of the concentration of velvet bean extract on the individual growth (length and weight) and gonadal development especially in fecundity and egg diameter of female tilapia. Thus, to determine the optimum concentration to increase growth and decrease the reproductive activity of velvet bean extract in female Nile tilapia.

MATERIALS AND METHODS

Research design

This study used an experimental method to determine the effect of the concentration of velvet bean extract on the individual growth and gonadal development of female tilapia. The research design used was a completely randomized design (CRD) with 5 treatments and each of them had 4 replications. The concentration of velvet beans extract used five different concentrations (P0) without velvet beans extract; (P1) velvet beans extract with a concentration of 1 $ml.kg^{-1}$ feed; (P2) velvet beans extract with a concentration of 3 $ml.kg^{-1}$ feed; (P3) velvet beans extract with a concentration of 5 $ml.kg^{-1}$ feed; (P4) velvet beans extract with a concentration of 7 $ml.kg^{-1}$ feed.

Extraction and application

The velvet bean seed extract was prepared using the maceration method using ethanol and air as solvents with a ratio of 1:1 (Yantika, 2015). The velvet bean seed is roasted to deactivate the anti-nutritional substances contained in the seeds, then pulverized using a food processor, and sifting is carried out so that the resulting velvet bean seed powder is smooth. A total of 150 g of velvet bean seed powder was extracted by maceration method in distilled water and ethanol with a volume of 375 ml: 375 ml, and this was done three times. The residue was removed, then the filtrate was removed and collected, then

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separated from the ethanol and concentrated in a rotary evaporator at 50 rpm, with a temperature of 55ºC with a water bath.

The velvet bean seed extract was given to the fish orally by mixing the extract with different treatments with distilled water to produce 70 ml of the extract solution and adding a binder in the form of CMC as much as 0.35 g as an adhesive and coating for the velvet bean seed extract with the feed. A commercial pellet Hi-Pro-Vite (781-2) as the basal diet was evenly sprayed with velvet beans seed extract and then dried.

Fish rearing

Fish maintenance was carried out for 60 days. The fish were fed with an experimental diet containing varying level of velvet beans seed extract. The amount of feed given ranges from 3 - 5% of fish body weight. Feed is given 2 times a day (Djunaedi *et al.*, 2016).

Parameters observed

The parameters observed in this study included the growth in length of individual fish, growth in weight of individual fish, fecundity, diameter of fish eggs, and water quality. Individual length and weight are weighed every ten days for 60 days, fecundity is measured by the gravimetric method, according to Effendie (1997) is formulated as follows:

$$F = \frac{G x f}{g}$$

Description:

F= Fecundity (eggs)

G = Total gonad weight (g)

f = Number of eggs in the gonad sub sample

g = Gonadal subsample weight (g)

Egg diameter measurements were carried out using histological preparations which were observed under a microscope and connected to the Optilab application so that the egg diameter will be automatically known. Water quality measurements include pH, temperature, dissolved oxygen, and ammonia levels were measured once every 10 days. Measurement of pH with pH paper, temperature and dissolved oxygen using a Lutron^{TM} DO meter, and ammonia levels using an ammonia test kit.

Data analysis

Data on growth in length, individual weight, fecundity and egg diameter will be processed using Analysis of Variance, followed by Duncan's Multiple Range Test, with the application of SPSS version 25. Meanwhile, the water quality parameters were analyzed descriptively based on relevant reference to get a scientific conclusion.

RESULTS AND DISCUSSIONS

Female tilapia fed with the addition of velvet bean seed extract at different concentrations resulted in growth in individual lengths ranging from 13.5-16.1 cm from the initial length of 5.3-5.6 cm (Figure 1). Velvet bean seed added at different concentrations had a significant effect on the length growth of individual female tilapia (P>0.05). The highest length in the 6th week was obtained from fish that were cultured with the addition of 5 ml.kg^{-1} of velvet beans seed extract of 16.1 cm. The lowest individual length in fish fed without the addition of velvet beans seed extract was 13.5 cm.

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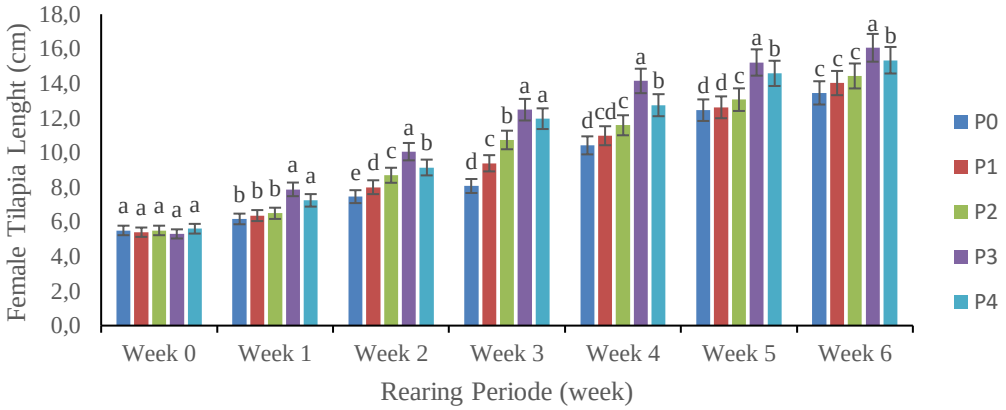

Figure 1. Growth of tilapia individual length during 60 days of rearing (based on sampling of 10% of the population) Different superscript letters (a, b, c) in the same column indicate that there is a significant difference from each treatment (P<0.05); P0 feed without supplementation; P1 velvet bean seed extract supplementation feed 1 ml.kg^{-1} feed; P2 velvet bean seed extract supplementation feed 3 ml.kg^{-1} feed; P3 feed with velvet bean seed extract supplementation 5 ml.kg^{-1} feed; P4 feed with velvet bean seed extract supplementation 7 ml.kg^{-1} feed.

Different concentrations of velvet bean seed extract added to the feed resulted in the growth of individual female tilapia weights ranging from 60.02 - 89.43 g (Figure 2). The addition of velvet bean seed extract with different concentrations had a significant effect on the growth of female tilapia individual weight (P>0.05). The highest weight in the 6th week was obtained from fish that were cultured with the addition of 5 ml.kg^{-1} of velvet bean seed extract of 89.43 g. The lowest individual weight of fish fed without the addition of velvet bean seed extract was 60.02 g. The fecundity of female tilapia feed with the addition of velvet bean seed extract at different concentrations ranged from 2,190.69 to 2,531.12 eggs (Table 1). The addition of velvet bean seed extract with different concentrations had a significant effect on the fecundity of female tilapia (P>0.05). The highest fecundity was obtained from fish that were cultured without the addition of velvet bean seed extract of 2,531.12 ± 6.78 eggs. The lowest fecundity was found in female tilapia fed with the addition of velvet bean seed extract 7 ml.kg^{-1} of feed of 2,190.58 ± 7.47 eggs. Female tilapia fed with the addition of velvet bean seed extract with different concentrations had egg diameters of 0.73 – 3.36 mm (Table 1). The addition of velvet bean seed extract with different concentrations had a significant effect on female tilapia egg diameter (P>0.05). The largest egg diameter was obtained from fish that were cultured without the addition of velvet bean seed extract of 3.06 mm. The smallest egg diameter was found in female tilapia which was fed with the addition of velvet bean seed extract 7 ml.kg^{-1} of feed by 0.83 mm.

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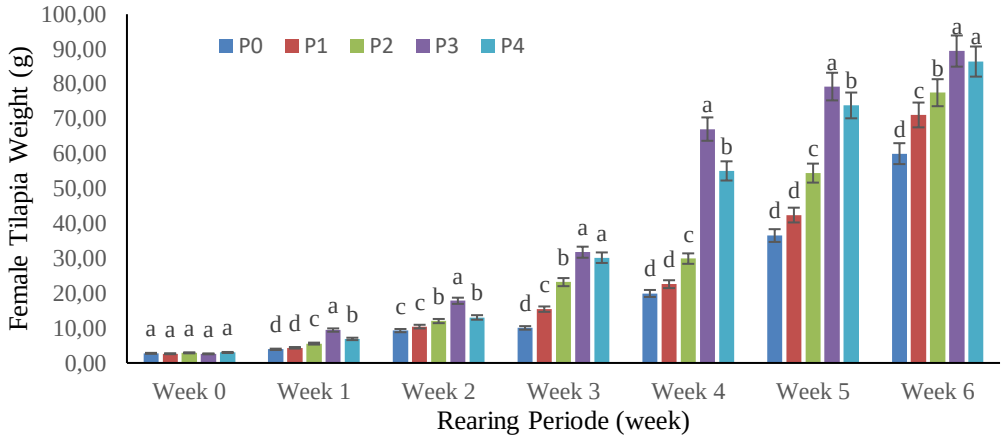

Figure 2. Growth of tilapia individual weight during 60 days of rearing (based on sampling of 10% of the population in every treatment) Different superscript letters (a, b, c) in the same column indicate that there is a significant difference from each treatment ($P<0.05$); P0 feed without supplementation; P1 velvet bean seed extract supplementation feed 1 ml.kg^{-1} feed; P2 velvet bean seed extract supplementation feed 3 ml.kg^{-1} feed; P3 feed with velvet bean seed extract supplementation 5 ml.kg^{-1} feed; P4 feed with velvet bean seed extract supplementation 7 ml.kg^{-1} feed.

Female tilapia reared for 60 days were fed with velvet bean seed extract treatment with different doses with temperatures ranging from 26.3 - 27.8°C in the morning and 28.6 - 29.5°C in the afternoon. The pH of the water ranges from 7-8 either in the morning or evening. The dissolved oxygen value during the morning is in the low range of 2.78 - 3.53 mg.L^{-1}. In the afternoon it increased between 5.43 - 6.33 mg.L^{-1}. Ammonia levels measured once a week ranged from 0 - 0.25 mg.L^{-1}. The results of water quality measurements during maintenance (Table 2). The addition of velvet bean seed extract to feed with different concentrations for female Jatimbulan tilapia had a significant effect on the growth of individual length, individual weight, fecundity, and egg diameter. Velvet bean seed contains 5.38 - 6.98% L-dopa, non-protein amino acids, and alkaloids (Bastiar *et al.*, 2017; Lampariello et al., 2012). L-Dopa is a non-protein amino acid, a precursor of dopamine which can stimulate GH secretion and inhibit GnRH release from the pituitary (Martinez *et al.*, 2017; Kamil and Bugar, 2013). L-Dopa in the brain will be synthesized into dopamine. High levels of dopamine will increase the somatic growth of tilapia through two pathways. Dopamine will bind to D1 and D2 receptors in the hypothalamus. First through D1 receptors that stimulate GH secretion, then D1 receptors also modulate glutamatergic neurons so that GnRH release will be inhibited. Second, dopamine will bind to D2 receptors on the pituitary nerve endings and directly inhibit the release of GtH cells from the pituitary. High levels of dopamine will increase the bond between receptors of dopamine. Many bonds between dopamine and D1 receptors will increase GH production, and more bonds with D2 receptors will suppress gonadotropin production (Shruti et al., 2018; Ilgin, 2020; Ganesh, 2021)

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Table 1. Fecundity and egg diameter of Nile tilapia after 60 days of rearing.

Treatment	Fecundity (Eggs) ± SD	Egg diameter (mm) ± SD
0 ml.kg^{-1} feed (P0)	2531.12±6.78^{a}	3.06±0.30^{a}
1 ml.kg^{-1} feed (P1)	2383.3±8.64^{b}	2.11±0.25^{b}
3 ml.kg^{-1} feed (P2)	2221.44±17.78^{c}	1.39±0.21^{c}
5 ml.kg^{-1} feed (P3)	2206.33±19.34^{cd}	1.11±0.16^{cd}
7 ml.kg^{-1} feed (P4)	2190.69±7.47^{d}	0.83±0.10^{d}

Different superscript letters (a, b, c) in the same column indicate there is a significant difference from each treatment (P<0.05).

Inhibited GnRH and GtH will make feed nutrition focused on somatic growth. GH will go into the fish's blood circulation and into the target organs such as the liver, muscles, and bones to support fish growth. GH also affects the processes of osmoregulation, nutrient metabolism, formation of bones and soft tissues, (Bjornsoon et al., 2018; Reinecke et al., 2005). The growth in length and weight of individuals increased in the treatment with the addition of velvet bean seed extract. Because at optimal doses the use of L-Dopa stimulated an increase in dopamine in the hypothalamus. Dopamine which binds to the D1 receptor will stimulate the secretion of growth hormone (GH). The content of L-dopa in velvet bean seeds stimulates the release of GH to stimulate the secretion of IGF-1 (insulin growth factor 1) so that the mesenchymal cells stimulate cell proliferation and produce bone and muscle growth which has an impact on increasing the value of the growth rate in length and weight (Loekman et al., 2018). However, an increase in the dose of the extract in the feed exceeding 5 ml.kg^{-1} feed showed a lower growth rate. At more than optimal doses, L-Dopa further increases GH, which will result in negative feedback. Excessive amounts of GH and IGF-I in the blood vessels will cause negative feedback in the form of impulses to the pituitary gland not to secrete GH. GH that exceeds the optimal limit provides negative feedback to the pituitary gland in two ways, namely directly being antagonistic so that GH secretion is inhibited and indirectly stimulating somatostatin so that GH work is inhibited (Apriliani, 2018).

Table 2. Water quality of Nile tilapia after 60 days of rearing

Treatment	Parameters						
	Temperature (^{o}C)		pH		DO (mg.L^{-1})		Ammonia (mg.L^{-1})
	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	
P0	26.7-26.8	28.8-29.5	7	7	3.01-3.53	5.43-6.21	0-0.25
P1	26.3-26.7	28.6-29.0	7	7-8	2.78-3.26	5.89-6.18	0-0.25
P2	26.5-27.1	28.9-29.2	7	7	2.94-3.11	5.72-6.03	0-0.25
P3	26.5-27.4	28.9-29.3	7	7-8	2.98-3.47	5.47-6.33	0-0.25
P4	26.9-27.8	29.0-29.5	7	7	2.79-3.03	5.58-6.15	0-0.25

The fecundity of fish fed with velvet bean seed extract showed the lowest fecundity obtained from fish fed with the addition of velvet bean seed extract of 7 ml.kg^{-1} of feed with a total of 2190 eggs, whereas in the treatment without the

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addition of velvet bean seed extract the fecundity of tilapia reached 2531 grain. Kara nuts contain L-Dopa. L-Dopa is a precursor of dopamine. Feed that is added to L-Dopa will be converted into dopamine with the help of the enzyme Dopa decarboxylase (DDC) (Lampariello et al., 2012). Dopamine will give a signal from the hypothalamus to inhibit the retention of GnRH which acts on the pituitary gland, so that FSH cannot synthesize the hormone testosterone into estrogen. The estrogen will produce 17β-estradiol which functions to stimulate the liver to synthesize vitellogenin which is then carried by the bloodstream to form oocytes (Nagahama et al., 1995). This is in accordance with this study, which states that fecundity decreases with each increase in velvet seed extract dose because the estrogen hormone cannot synthesize vitellogenin optimally so egg yolk protein does not form.

The addition of velvet bean seed extract with different doses in feed on female tilapia culture also affects egg diameter. The higher the dose given, the smaller the diameter of the fish eggs. In the treatment without the addition of extra velvet bean seed, the diameter of female tilapia reached 3.06 mm. Meanwhile, with the addition of a dose of velvet bean seed extract 7 $ml.kg^{-1}$ the diameter of female tilapia egg feed reached 0.83 mm. Gonadotropin hormones that are inhibited by dopamine lead to the inhibition of GnRH retention (Fontaine et al., 2013). So FSH cannot synthesize the hormone testosterone into estrogen which produces 17β-estradiol. Decreased estradiol causes liver stimulation for the vitellogenesis process to be inhibited, then causes oocyte size to decrease (Yuniarti et al., 2021). This is consistent with this study, which stated that the diameter of tilapia eggs decreased with each increase in the dose of velvet bean seed extract, due to low estradiol levels inhibiting the vitellogenesis process resulting in lower oocyte formation.

In the process of cultivating tilapia, the temperature is 26.3 to 29.5 ºC in the morning and evening. This range is optimal for rearing tilapia. The pH ranges from 7 - 8 both during the day and evening. The pH is optimal enough for fish growth. Optimal temperature and pH will support good fish growth (Fauzia and Suseno, 2020). Dissolved oxygen levels reach 2.78 $mg.L^{-1}$ in the morning and reach 6.33 mgL^{-1} in the afternoon. Ammonia levels range from 0 $mg.L^{-1}$ to 0.25 $mg.L^{-1}$. Dissolved oxygen and ammonia levels are good for the life of tilapia (Pramleonita et al., 2018). Water quality in optimal tilapia culture does not affect fecundity, egg diameter, and gonadal maturity level. So that the three parameters are actually affected by the treatment of adding velvet bean seed extract at different doses.

CONCLUSION

The use of different concentrations of velvet bean seed extract affected individual growth (length and weight) and gonadal development (fecundity and egg diameter) of female tilapia. The use of velvet bean seed extract at a concentration of 5 $ml.kg^{-1}$ of feed resulted in better growth and decreased fertility and egg diameter. In this study the optimum concentration of velvet bean seed extract 5 $ml.kg^{-1}$ of feed resulted in the highest growth in individual length of 16.1 cm and individual weight of 89.43 g, reducing fecundity to 2,206 eggs and egg diameter to 1.11 mm.

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