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Evaluation of melastomamelastoma *Melastoma malabathricum* leaf extract in the gonad development and growth performance of Nile tilapia *Oreochromis niloticus*

Evaluasi pemberian ekstrak daun melastoma *Melastoma malabathricum* terhadap perkembangan gonad dan kinerja pertumbuhan ikan nila *Oreochromis niloticus*

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ABSTRACT

The objective of this study was to determine the optimum dose of melastoma *Melastoma malabathricum* leaf extract to inhibit the gonad development of Nile tilapia, increase its growth rate, and evaluate its effectivity as natural sex reversal agent. This study used a completely randomized design containing five extract dose treatments (0, 0.5, 1, 2, and 4 g/kg diet doses) and three replications. The undifferentiated Nile tilapia larvae (7 days post hatching) were randomly distributed to fifteen aquaria and maintained for 112 days using a recirculation system. The results showed that all dose treatments were not significantly different (P>0.05) in gonadosomatic index values of the D84 and D98 samplings. However, the 1 g/kg diet (D112) was significantly different (P<0.05) in all dose treatments. The final histological results (D112) showed that the 1 g/kg diet obtained the highest inhibition level of the testis and ovary developments, which were still in stage II compared to 0.5 g/kg diet (stage III) and control (stage IV and V). The highest average weight, absolute growth rate, and specific growth rate were obtained in the 1 g/kg diet dose which was significantly different (P<0.05) compared to the control. The percentage of males increased significantly following the increased dose treatment fed to the fish (4 g/kg diet) with 80.12 ± 4.67%, but the survival rate significantly decreased compared to the control. The administration of 1 g/kg diet dose obtained the best dose and potential as an inhibitor of gonad development in Nile tilapia.

Keywords: *Melastoma malabathricum*, gonad inhibition, cytosterol, *Oreochromis niloticus*

ABSTRAK

Tujuan penelitian ini menentukan dosis optimum ekstrak daun melastoma *Melastoma malabathricum* untuk menghambat perkembangan gonad, meningkatkan laju pertumbuhan, dan mengevaluasi efektivitasnya sebagai agen seks reversal alami. Penelitian ini menggunakan rancangan acak lengkap dengan lima perlakuan (dosis 0; 0,5; 1,0; 2,0; dan 4,0 g/kg pakan) dan tiga ulangan. Larva ikan nila sebelum kelamin terdiferensiasi (7 hari pascatetas) secara acak dimasukkan ke dalam 15 buah akuarium dan dipelihara selama 112 hari pada sistem resirkulasi. Hasil menunjukkan bahwa semua perlakuan tidak berbeda nyata (P>0,05) terhadap nilai GSI pada sampling D84 dan D98. Namun, perlakuan 1 g/kg pakan pada sampling D112 berbeda nyata dengan semua perlakuan. Hasil histologi terakhir (D112) menunjukkan bahwa perlakuan 1 g/kg pakan mengindikasikan penghambatan perkembangan testis dan ovari yang paling besar yang masing-masing berada pada TKG II, dibandingkan dengan perlakuan 0,5 g/kg pakan (TKG III), dan dibandingkan dengan kontrol (TKG IV dan TKG V). Pengamatan terhadap bobot rata-rata, laju pertumbuhan mutlak, dan laju pertumbuhan harian tertinggi diperoleh pada perlakuan 1 g/kg pakan yang berbeda nyata (P<0,05) dibandingkan dengan kontrol. Persentase jantan meningkat secara signifikan (P<0,05) seiring meningkatnya konsentrasi ekstrak yang mencapai 80,12 ± 4,67% pada perlakuan 4 g/kg pakan, namun tingkat kelangsungan hidup menurun dibandingkan kontrol. Pada keseluruhan parameter, pemberian ekstrak 1 g/kg pakan merupakan dosis terbaik dan potensial sebagai penghambat perkembangan gonad pada ikan nila.

Kata kunci: *Melastoma malabathricum*, penghambatan gonad, sitosterol, *Oreochromis niloticus*

INTRODUCTION

Nile tilapia is one of the widely distributed and cultured freshwater commodities with high economical value, environmentally tolerant, high survival rate, and dimorphism sex characteristic (Junior *et al*., 2017; Kapinga *et al*., 2018). The monosex culture of male Nile tilapia is widely performed due to the mixed-sex culture often occurs a fast gonad development (Abdelhak *et al*., 2013) and uncontrolled spawning (Mbiru *et al*., 2015; Wahyuningsih *et al*., 2018). This causes the inhibited growth, and the individual size in population is uniformed (Omitoyin *et al*., 2013). This occurs as the allocated energy for somatic growth is used for gonad development (Kapinga *et al*., 2018), therefore taking a long period to reach the consumed size. Moreover, the male Nile tilapia growth is faster than the female Nile tilapia (Bhatta *et al*., 2012; Fuentes-Silva *et al*., 2013), which makes the mixed-culture occurs a competition between male and female fish to obtain food (Omitoyin *et al*., 2013).

Several methods developed to maintain the Nile tilapia reproduction are sex reversal, selection, hybridization, or genetic manipulation (Abdelhak *et al*., 2013; Gabriel *et al.,* 2017). Among these methods, the sex reversal technique is the most popular method by using the 17α-metiltestosteron synthetic steroid hormone (Ramirez *et al*., 2017). However, the usage of this hormone is strictly limited due to the negative impact in the environment, health, relatively expensive (Obaroh & Achionye-Nzeh, 2011; Gabriel *et al.*, 2017). Therefore, it needs another effort with the same objective as to inhibit the gonad development using a phytosteroid hormone obtained from herbal plant extract. The herbal plant is considered as the best solution to maintain the gonad development in Nile tilapia culture due to safer, easily obtained, more economical, and more environmentally friendly, which tends to easily degradable (Obaroh & Achionye-Nzeh, 2011; Gabriel *et al*., 2015; Ghosal & Chakraborty, 2017). Phytosteroids have been reported to inhibit the gonadal development of Nile tilapia. Jegede (2010) reported the *Hibiscus rosasinensis* extract at 2 g/kg diet dose could degrade testis and ovary of Nile tilapia, while Kushwaha (2013) also reported the similar results after the *Aloe vera* extract at 1 g/kg diet dose supplementation in Nile tilapia.

One potential plant that has steroidogenic characteristics is melastoma *Melastoma malabathricum* L. This plant is a wild plant and widely abundant in all tropical regions, mainly in the humid regions, namely, mountain slopes, thorns, and nearly dried field (Rajenderan, 2010). This plant is known to the public as a traditional medicinal plant for uterine recovery after birth, strengthening the uterus, wound healing acceleration, relieving post-menstrual syndrome, vaginal discharge, and fertility improvement (Joffry *et al*., 2012). Faravani (2009) reported that melastoma leaves contain flavonoids, saponnins, tannins (glycosides), and steroids or triterpenoids. Farizah *et al*. (2017) showed that the melastoma leaf extract in the optimum dose could induce the gonad maturation of crab *Scylla olivacea*, but the high dose will inhibit the gonad development. The melastoma leaf extraction with ethanol produces three bioactive compounds at >90% purity level, namely, squalenes, α-tocopherol, and cytosterols, which act as a stimulator (aphrodisiac) or inhibitor.

The objective of this study was to determine the optimum dose melastoma leaf extract that could inhibit the Nile tilapia gonad development, therefore improving the somatic growth and evaluating its potential as a natural sex reversal agent.

MATERIALS AND METHODS

Experimental materials

The study was performed in October, 2019 and February, 2020 in the Zaki fish farm, Ciampea, Bogor. The materials used in this study were undifferentiated Nile tilapia larvae (7 day after hatching, dah) with 0.012 ± 0.001 g weight and 0.87 ± 0.004 cm lengths and obtained from the Experimental Ponds, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University, Bogor. The melastoma leaves used as an extraction material were fresh green leaves (third to fifth leaf part from the shoots) originated from Mentawai Islands, West Sumatra. These young leaves were considered to physiologically ripe, and the phytochemical compound contents expected could work in the experimental fish at the optimum limit compared to the old leaves which were likely to have higher toxic effects.

Study design

This study used a completely randomized design containing five treatment groups. Each treatment had three replications randomly (Table 1).

Table 1. Melastoma leaf extract supplementation in diet treatment groups.

Treatment	Note
Control	Diet without melastoma leaf extract
0.5 g/kg diet	Diet with 0.5 g/kg diet melastoma leaf extract dose
1 g/kg diet	Diet with 1 g/kg diet melastoma leaf extract dose
2 g/kg diet	Diet with 2 g/kg diet melastoma leaf extract dose
4 g/kg diet	Diet with 4 g/kg diet melastoma leaf extract dose

Melastoma leaf extract

The melastoma leaf extraction with ethanol followed the procedure of Balamrugan *et al*. (2013). The phytochemical test was performed following the Harborne (1987) method, and the characterization process to identify the bioactive compounds in the melastoma leaf extract was performed by the GC-MS test referred to Rahmwati (2008).

Experimental fish and diet preparation

The Nile tilapia larvae were acclimatized for three days in two units of the 100 cm × 50 cm×50 cm^3 aquarium, which were previously cleaned. During the acclimatization process, fish were fed three times a day in satiation with a commercial diet containing 41% protein. After acclimatization, the initial weight and length were measured. Fish were distributed to the treatment aquaria with 1 fish/6 L water (SNI-6141, 2009) or 30 fish/aquarium. The treatment diets were made by mixing the melastoma leaf extract in the commercial diet following the treatment dose. Mixing was performed by the coating method using a sprayer as adopted from the diet mixed with methyltestosterone (MT).

Gonad inhibition treatment

The undifferentiated Nile tilapia (7 dah) were maintained in 15 aquarium units (100×50×50 cm^3) for 112 days which obtained the water supply from a recirculation system. Larvae were fed with a commercial diet (CP 41%) sprayed with melastoma leaf extract following the dose treatments provided. During the maintenance period, pH and temperature were measured every day, DO (dissolved oxygen) was measured every week, and total ammonia (total ammonia nitrogen, TAN) was measured at the initial and end of maintenance period. Feces and feed waste were syphoned during the maintenance period.

Experimental parameters

The GSI measurement and gonad histology identification were performed at the 84th, 98th, and 112th day of maintenance period by taking two fish per aquarium. The gonad histology was performed using the Haematoxiline-Eosine and GSI was calculated following the Abdelhak *et al.* (2013) formula:

$$\text{GSI (\%)} = \frac{\text{Gonad weight (g)}}{\text{Body weight (g)}} \times 100$$

The visual sex ratio calculation was performed by taking all fish from each treatment at the end of the study. This identification process was performed by directly observing the genital morphologically with the methylene blue. The male sex ratio was calculated following Gabriel *et al*. (2017) formula:

$$\text{MSR (\%)} = \frac{\text{Total male fish}}{\text{Total observed fish}} \times 100$$

The calculation of absolute weight growth rate based on Kapinga *et al*. (2018) formula:

$$\text{AGR} = \text{Wt-Wo/t}$$

Note:
LPM : Absolute weight growth rate (g/day)
Wt : Final average weight (g)
Wo : Initial average weight (g)
t : Maintenance period

The daily weight growth rate (α) observation in the final maintenance period used Kapinga *et al*. (2018) formula:

$$\text{SGR (\%/day)} = \left[\sqrt[t]{\frac{\text{Wt}}{\text{Wo}}} - 1\right] \times 100$$

Note:
LPH : Daily weight growth rate (%/day)
Wo : Initial average weight (g)
Wt : Final average weight in t-period (g)
t : Maintenance period

The survival rate of the experimental fish was obtained from the percentage of living fish in the end of maintenance period with the total fish on the initial maintenance period. The survival rate was calculated using the Kapinga *et al*. (2018) formula:

$$SR\ (\%) = \frac{N_t}{N_0} \times 100$$

Note:
SR : Survival rate (%)
Nt : Total fish on t-day (fish)
No : Total fish on 0-day (fish)

The feed conversion ratio was calculated based on the Kapinga *et al*. (2018) formula:

$$FCR = \frac{F}{[(Bt + Bm) - Bo]}$$

Note:
B_t : The fish biomass in the final maintenance period
B_m :The dead fish biomass during maintenance period
B_0 : The fish biomass in the initial maintenance period
F : Total consumed diet during maintenance period

During the maintenance period, pH and temperature were measured every day, DO (dissolved oxygen) was measured every week, and total ammonia (total ammonia nitrogen, TAN) was measured at the initial and end of the maintenance period.

Data analysis

The results obtained were analyzed descriptively by using the Microsoft Excel 2013 and statistically using the analysis of variance (ANOVA) with SPSS 22.0 at 95% confidence level. When there was a significant difference among treatment groups ($P<0.05$), the statistical analysis was continuously performed with the Duncan test at $\alpha=0.05$.

RESULTS AND DISCUSSION

Results

The highest Nile tilapia survival rate was obtained in the control treatment group (93.33 ±

Table 2. Survival rate (SR), feed conversion ratio (FCR), and male sex ratio (MSR) of Nile tilapia fry after feeding the melastoma leaf ethanolic extract for 112 days.

Treatment	SR (%)	FCR	MSR (%)
Control	93.33 ± 3.34^{b}	1.27 ± 0.10^{c}	66.67 ± 7.22^{a}
0.5 g/kg diet	90.00 ± 3.33^{ab}	1.01 ± 0.08^{ab}	74.18 ± 5.72^{ab}
1 g/kg diet	90.00 ± 3.33^{ab}	0.91 ± 0.08^{a}	77.75 ± 0.82^{b}
2 g/kg diet	85.55 ± 5.09^{ab}	1.00 ± 0.01^{ab}	79.23 ± 1.50^{b}
4 g/kg diet	84.44 ± 5.09^{a}	1.09 ± 0.11^{b}	80.11 ± 4.67^{b}

The similar superscript letters in the same column showed an insignificant different (P>0.05) (Duncan's multiple range test).

Table 3. Nile tilapia growth performance after feeding the melastoma leaf ethanolic extract for 112 days.

Treatment	Weight (g)	Length (cm)	AGR (g/day)	SGR (%/day)
Control	35.39 ± 1.81^{a}	12.62 ± 0.28^{a}	0.31 ± 0.,02^{a}	7.42 ± 0.07^{a}
0.5 g/kg diet	44.10 ± 3.29^{b}	13.62 ± 0.33^{cd}	0.40 ± 0.03^{b}	7.60 ± 0.06^{bc}
1 g/kg diet	51.19 ± 4.57^{c}	14.01 ± 0.40^{d}	0.46 ± 0.04^{c}	7.74 ± 0.08^{c}
2 g/kg diet	43.16 ± 2.62^{b}	13.20 ± 0.30^{ab}	0.39 ± 0.04^{b}	7.59 ± 0.12^{bc}
4 g/kg diet	38.22 ± 3.96^{ab}	12.83 ± 0.27^{ab}	0.34 ± 0.04^{ab}	7.46 ± 0.12^{ab}

The similar superscript letters in the same column showed an insignificant different (P>0.05) (Duncan's multiple range test). AGR (absolute growth rate) and SGR (specific growth rate).

Table 4. The GSI value of Nile tilapia fry after feeding the melastoma leaf ethanolic extract for 112 days

Treatment	GSI D84	GSI D98	GSI D112
Control	0.22 ± 0.01^{a}	0.42 ± 0.36^{a}	0.45 ± 0.02^{c}
0.5 g/kg diet	0.19 ± 0.14^{a}	0.30 ± 0.05^{a}	0.36 ± 0.03^{b}
1 g/kg diet	0.10 ± 0.01^{a}	0.27 ± 0.03^{a}	0.28 ± 0.05^{a}
2 g/kg diet	0.17 ± 0.07^{a}	0.36 ± 0.10^{a}	0.37 ± 0.05^{b}
4 g/kg diet	0.18 ± 0.04^{a}	0.39 ± 0.16^{a}	0.45 ± 0.03^{c}

The similar superscript letters in the same column showed an insignificant different (P>0.05) (Duncan's multiple range test). D84 (84th day), D98 (98th day), and D112 (112th).

3.34%), that was significantly different (P<0.05) from the 4 g/kg diet treatment group, which obtained the lowest survival rate value (84.44 ± 5.09%; Table 2). The highest feed conversion rate was obtained in the control treatment group at 1.27 ± 0.10, that was significantly different (P<0.05) from the 1 g/kg diet treatment group at 0.91 ± 0.08 as the lowest feed conversion ratio value (Table 2). The highest male sex ration reached 80.12 ± 4.67% in the 4 g/kg diet treatment group, that was significantly different (P<0.05) from the control treatment group with 66.68 ± 7.22% as the lowest male sex ratio value (Table 2).

The highest Nile tilapia growth performance (average weight, length, absolute growth rate, and specific growth rate) was obtained from the 1 g/kg diet treatment group at 51.19 ± 4.57 g; 14.01 ± 0.40 cm; 0.46 ± 0.04 g/day; and 7.74 ± 0.08 %/day, respectively, and significantly different (P<0.05) from the control treatment group at 35.39 ± 1.81 g; 12.62 ± 0.17 cm; 0.31 ± 0.02 g/day; and 7.42 ± 0.07 %/day as the lowest values (Table 3).

The highest GSI value at the 84th and 98th day of maintenance period were obtained from the control treatment group at 0.22 ± 0.01% and 0.42 ± 0.36%, respectively, while the lowest value was obtained from the 1 g/kg diet treatment group at 0.11 ± 0.01% and 0.28 ± 0.03%, respectively (Table 4). The continuous test showed that all treatment groups were insignificantly different (P>0.05). However, the GSI value at the 112th day of maintenance period in the 1 g/kg diet treatment group was significantly different (P<0.05) in all treatment groups (Table 4). During the maintenance period, the water quality was maintained at the tolerance limit for the experimental fish (Table 5).

Table 5. Water quality range of Nile tilapia maintenance during the study.

Parameter	Water quality range	Optimum value
DO	4.10–6.8 mg/L	>4.0 mg/L (Manduca *et al.*, 2020)
TAN	0.01–0.977 mg/L	≤0.2 mg/L (Manduca *et al.*, 2020)
pH	6.5–7.6	6.5–8.5 (Manduca *et al.*, 2020)
Temperature	26°C – 29°C	25°C–30°C (Larasati *et al.*, 2020)

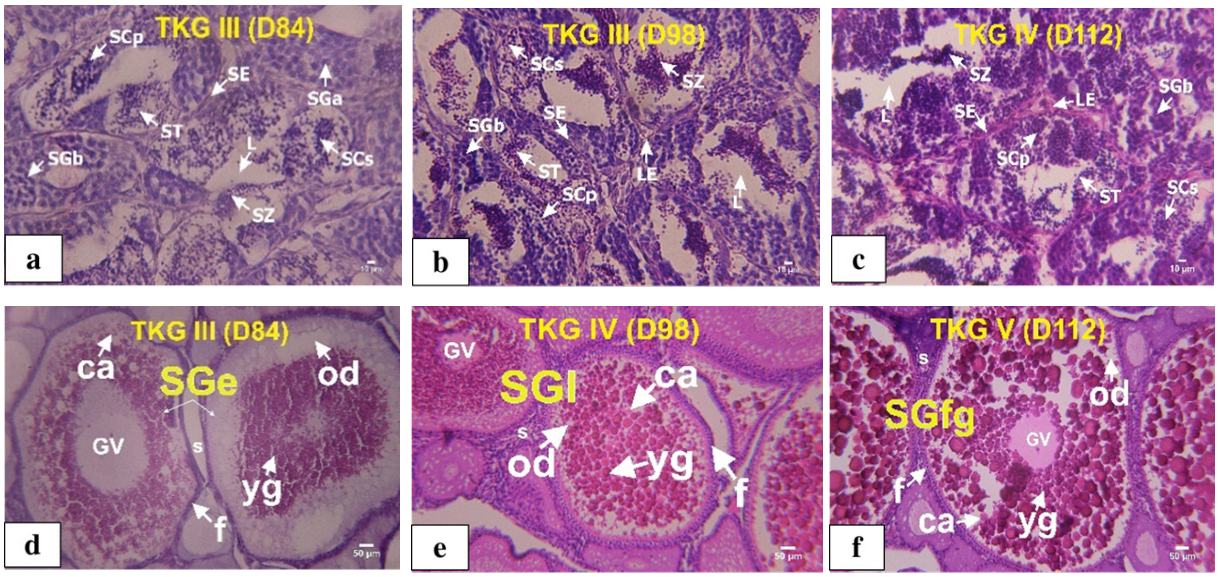

Figure 1. Testis (a, b, c) and ovary (d, e, f) histological results of Nile tilapia in the control treatment group. Note: a) testis histology (D84) in stage III dominated by the secondary spermatocytes (SCs) and spermatids (ST), also the closed occurence stage of spermatozoa (SZ); b) testis histology (D98) in stage III dominated by the secondary spermatocytes (SCs), spermatids (ST), and abundant spermatozoa (SZ); c) testis histology (D112) in stage IV dominated by abundant spermatids (ST) dan spermatozoa (SZ); d) ovary histology in stage III (D84) in the early secondary growth step (SGe); e) ovary histology in stage IV (D98) in the late secondary growth steps (SGl); and f) ovary histology in stage V (D112) TKG V in the full-grown steps of secondary growth stage (PGfg). Lumen (L), type A spermatogonia (SGa), Leydig cells (LE), Sertoli cells (SE), Stroma (S), oil droplet (od)/lipid droplet, cortical alveoli (ca), and yolk globules (yg) (HE staining, bar scale: (a, b, c) 10 µm 100x magnification; (d, e, f) 50 µm 40x magnification).

Based on the gonad histology in the control treatment group, the structure and stage of gonad development was in the normal condition, namely, testis was at stage III (D84 and D98) and stage IV (D112); and ovary was at stage III (D84), stage IV (D98), and stage V (D112) (Figure 1). Compared to the histology results in the 0.5 g/kg diet treatment group obtained the testis and ovary histologies at stage I (D98), stage II (D98), and stage III (D112), and then the testis structure damage was only found in D84 (Figure 2). Furthermore, the 1 g/kg diet treatment group showed stage I (D84 and D98) with the damaged testis structure, namely, atrophy and degradation, which caused an empty spermatogenic part, and stage II (D112) with the severe damage in the testis structure (atrophy, tubulus seminiferus disintegration, and hydrophic degeneration). Meanwhile, the ovary histology was at the stage I (D84) and stage II (D98 and D112) (Figure 3). The histological results in the 2 g/kg diet treatment group showed the relatively similar testis histology to the control treatment group, but this treatment group occurred the testis structure damage (atrophy and degradation), namely, at the D84 (stage II), D98 (stage III), and D112 (stage IV). Meanwhile, the ovary development stage in the control treatment group was similar to the 0.5 g/kg diet treatment group, namely, stage I (D84), stage II (D98), and stage III (D112) (Figure 4). Furthermore, the testis and ovary histologies in the 4 g/kg diet treatment group entirely showed the gonad development that was relatively similar to the 2 g/kg diet treatment group and testis damage (atrophy and degradation) only in D84 (Figure 5). The comparison of testis and ovary histology results from the three observational sampling points (D84, D98, and D112) in each treatment group (control; 0.5; 1; 2; and 4 g/kg diets) can be seen in Table 6.

Discussion

The results showed that higher extract dose of melastoma leaf extract supplementation obtained a lower survival rate, mainly the survival rate value in 4 g/kg diet. This condition occurred due to the increased toxicity accepted by fish along with the increased extract dose supplementation, as presented in Gabriel *et al*. (2017). Farizah *et al*. (2017) reported that the melastoma leaves contain phytochemicals, namely, flavonoids, saponnins, tannins (glycosides), phenolics, steroids,

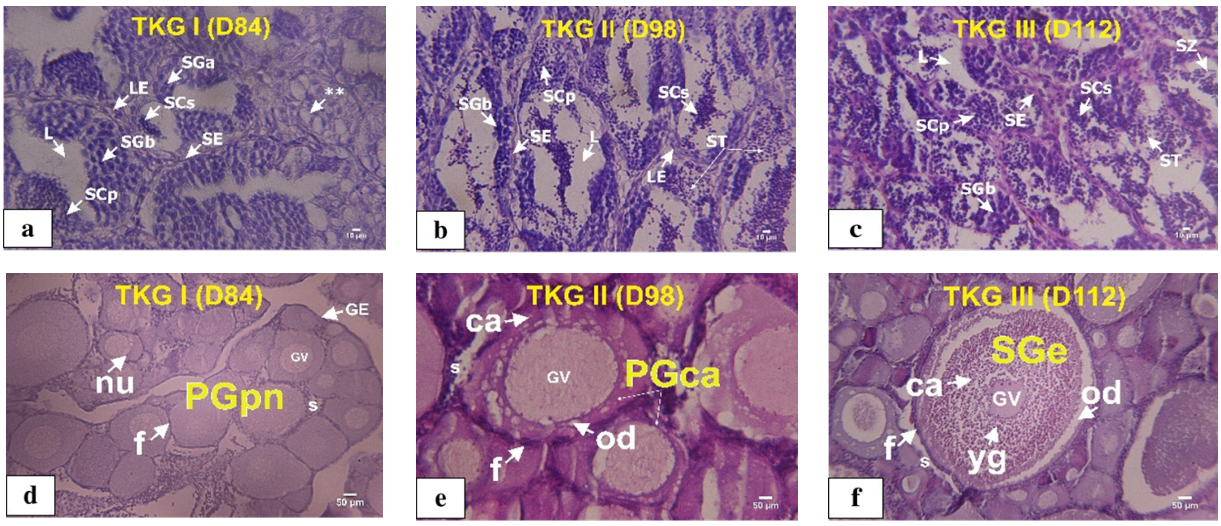

Figure 2. Testis (a, b, c) and ovary (d, e, f) histological results of Nile tilapia in the 0.5 g/kg diet treatment group. Note: a) testis histology (D84) in stage I dominated by the type B spermatogonia (SGb) and primary spermatocytes (SCp), closely heading to the secondary spermatocytes (SCs) phase with testicule tubular atrophy & empty spermatogenic cyst; b) testis histology (D98) in stage II dominated by the type B spermatogonia (SGb), secondary spermatocytes (SCs), and less spermatids (ST), c) testis histology (D112) in stage III dominated by the type B spermatogonia (SGb), secondary spermatocytes (SCs), spermatids (ST), and less spermatozoa (SZ); d) ovary histology (D84) in stage I in the Perinucleolar step of primary growth stage (PGpn); e) ovary histology (D98) in stage II in the Cortical alveoli step of primary growth stage (PGca); f) ovary histology (D112) in stage III in the Early secondary growth step (SGe). Lumen (L), type A spermatogonia (SGa), Leydig cells (LE), Sertoli cells (SE), Stroma (S), nucleoli (nu), oil droplet (od)/lipid droplet, cortical alveoli (ca), germinal vesicle (GV), follicular cells (f), germinal epithelium (GE), ** testicule tublar atrophy & empty spermatogenic cyst and yolk globules (yg) (HE staining, bar scale: (a, b, c) 10 µm 100x magnification; (d, e, f) 50 µm 40x magnification).

and triterpenoids. According to Mandalla *et al*. (2013), saponnins are toxic anti-nutrient compounds, mainly in diet from the plant protein; therefore, the supplementation in the fish culture is limited. Saponnins can improve the mucose cell permeability of the small intestines (Glencross, 2016). This extract also contains tannins which are known as anti-nutrient compounds from the polyphenol compounds that can inhibit the protein digestive enzyme activity (Glencross, 2016). Polyphenols can also precipitate protein by forming the cross-bonding between protein and other macromolecules, therefore unavailable in the digestive system (Mandalla *et al*., 2013).

Although saponnin compounds are toxic or anti-nutrient agent for fish, specifically in a high dose supplementation, these compounds are reported to induce the male sex production in Nile tilapia. The results showed the higher dose supplemented, the higher male sex ratio obtained, reaching to 80.12±4.67% in 4 g/kg diet treatment group (Table 2). These results were similar to Omitoyin *et al*. (2013); Ghosal *et al*. (2015); and Gabriel *et al.* (2017) in other plants, who reported that the percentage of male production in Nile tilapia increased along with the increased concentration of *T. Terrestris* and *Aloe vera*. One of the saponnin steroids action is to inhibit the aromatase enzyme (Gabriel *et al*., 2017). Moreover, the GC-MS test results showed that the melastoma leaf extract contains bioactive compounds, namely, *neophytadiene*, cytosterols, 9,12-*octadecadinoic acid* (linolelaidic acid), *hexadecanoic acid* (palmitic acid), *octadecadinoic acid* (linoleic acid), squalenes, and α-tocopherol with >90% purity level. One of the bioactive

Table 6. The histological comparison among the treatment groups (control; 0,5; 1; 2; and 4 g/kg diet)

Treatment	Histology	Day	Result	Structure	Characteristic
Control	Testis	84 & 98	Stage III	Normal	Dominated by the SCs, ST, and less SZ
		112	Stage IV	Normal	Mostly dominated by the ST and SZ
	Ovary	84	Stage III (SGe)	Normal	Visible ca, od were formed
		98	Stage IV (SGl)	Normal	Enlarged ca, od, and yg, and closely filled the oocyte
		112	Stage V (SGfg)	Normal	Large ca, od, and yg fully filled the oocyte
0.5 g/kg diet	Testis	84	Stage I	Testicule tubular atrophy & *empty spermatogenic cyst*	Dominated by the SGb, SCp, and closely headed to the SCs phase
		98	Stage II	Normal	Dominated by the SGb, SCs, and less ST
		112	Stage III	Normal	Dominated by SGb, SCs, ST, and less SZ phases
	Ovary	84	Stage I (PGpn)	Normal	Small ovarium with visible perinuclear
		98	Stage II (Pgca)	Normal	Formed ca and od
		112	Stage III (SGe)	Normal	Visible ca, od, and yg
1 g/kg diet	Testis	84 & 98	Stage I	Testicule tubular atrophy & *empty spermatogenic cyst*	Dominated by the SGb, less SCp, and SCs
		112	Stage II	Severe atrophy, tubulus seminiferus disintegration, and hydrophic degeneration	Dominated by the SGb, SCs, and less ST
	Ovary	84	Stage I (PGpn)	Normal	Small ovary and only visible hanya terlihat perinuklear
		98 & 112	Stage II (Pgca)	Normal	ca and od were formed

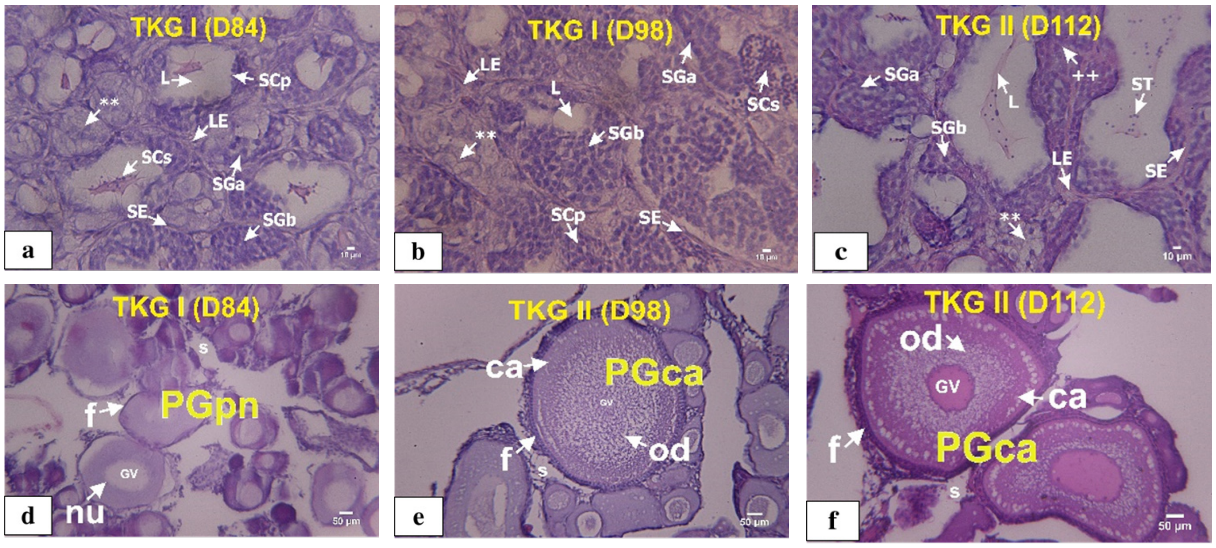

Figure 3. Testis (a, b, c) and ovary (d, e, f) histological results of Nile tilapia in the 1 g/kg diet treatment group. Note: a,b) testis histology (D84 & D98) in stage I dominated by the type B spermatogonia (SGb), and closely headed to the primary spermatocytes (SCp) and secondary spermatocytes (SCs) phases with testicule tubular atrophy & empty spermatogenic cyst; c) testis histology (D112) in stage II dominated by the type B spermatogonia (SGb), secondary spermatocytes (SCs), and closely headed to the spermatids (ST) phase with atrophy, tubulus seminiferus disintegration, and hydrophic degeneration; d) ovary histology (D84) in stage I in the Perinucleolar step of primary growth stage (PGpn); and e,f) ovary histology (D98 &D112) in stage II in the Cortical alveoli step of primary growth stage (PGca). Lumen (L), type A spermatogonia (SGa), Leydig cells (LE), Sertoli cells (SE), Stroma (S), nucleoli (nu), oil droplet (od)/lipid droplet, cortical alveoli (ca), germinal vesicle (GV), follicular cells (f), germinal epithelium (GE), ** atrofi tubular testikular &empty spermatogenic cyst, ++ hydrophic degeneration, and yolk globules (yg) (HE staining, bar scale: (a,b,c) 10 µm 100x magnification; (d,e,f) 50 µm 40x magnification).

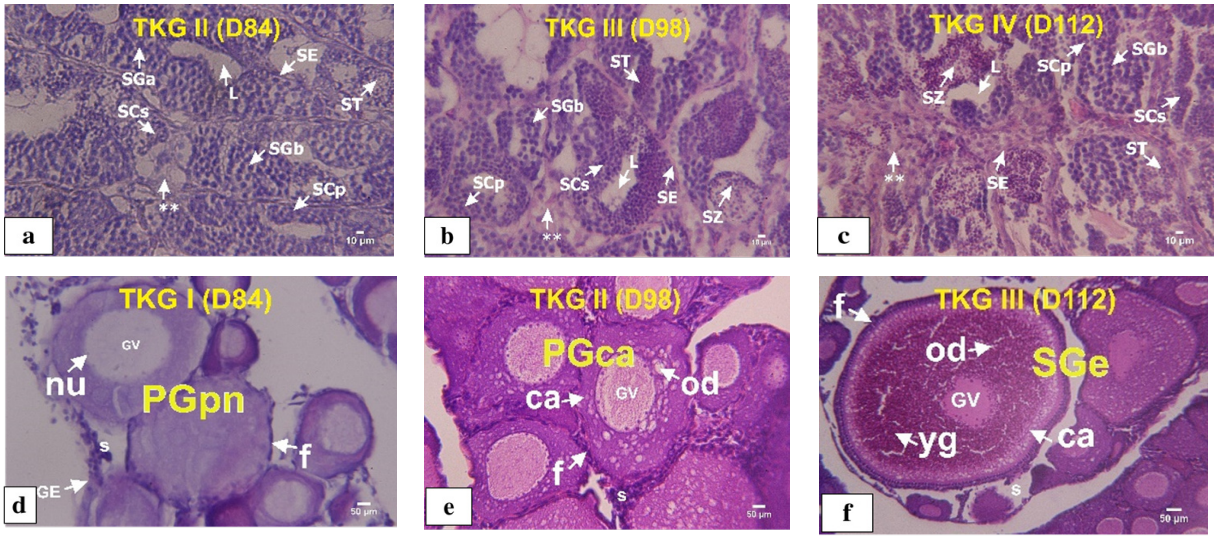

Figure 4. Testis (a, b, c) and ovary (d, e, f) histological results of Nile tilapia in the 2 g/kg diet treatment group. Note: a) testis histology (D84) in stage II dominated by type B spermatogonia (SGb), secondary spermatocytes (SCs), and less spermatids (ST) with testicule tubular atrophy & empty spermatogenic cyst; b) testis histology (D98) in stage III dominated by the type B spermatogonia (SGb), secondary spermatocytes (SCs), spermatids (ST), and less spermatozoa (SZ) with testicule tubular atrophy & empty spermatogenic cyst; c) testis histology (D112) in stage IV dominated by the type B spermatogonia (SGb), spermatids (ST), and abundant spermatozoa (SZ) with testicule tubular atrophy & empty spermatogenic cyst; d) ovary histologi (D84) in stage I in the Perinucleolar step of primary growth stage (PGpn); e) ovary histology (D98) in stage II in the Cortical alveoli step of primary growth stage (PGca); f) ovary histology (D112) in stage III in the Early secondary growth step (SGe). Lumen (L), type A spermatogonia (SGa), Leydig cells (LE), Sertoli cells (SE), Stroma (S), nucleoli (nu), oil droplet (od)/ lipid droplet, cortical alveoli (ca), germinal vesicle (GV), follicular cells (f), germinal epithelium (GE), **testicule tubular atrophy & empty spermatogenic cyst, and yolk globules (yg) (HE staining, bar scale: (a, b, c) 10 µm 100x magnification; (d, e, f) 50 µm 40x magnification).

compounds that may induce the Nile tilapia masculinity is cytosterol compounds through the aromatase enzyme inhibition (Rakel, 2018). The aromatase enzyme inhibitory activity causes the reduced estrogen synthesis (17β-estradiol) and induced androgen (testosterone) synthesis that leads to the male sex differentiation (Omitoyin *et al*., 2013; Ghosal & Chakraborty, 2017). Several plants have been reported to induce the masculinity in Nile tilapia, namely, *Mucuna pruriens* (Mukherjee *et al*., 2015), *Butea superb* (Kiriyakit, 2014), and seed ethanolic extract of *T. terrestris* (Ghosal & Chakraborty, 2017).

The study results showed that the melastoma leaf extract supplementation with the optimum dose improved the diet utilization effectiveness. This condition was occurring from the lower feed conversion ratio, mainly at 1 g/kg diet, than that from the other treatments (Table 2). In the similar dose treatment group, the growth performance, containing weight, length, absolute growth rate, and specific growth rate was higher than the control treatment group (Table 3). According to Gabriel *et al*. (2015), the herbal plant extract could increase the growth and diet utilization in Nile tilapia. The results were similar to Kareem *et al*. (2016), who utilized the *Cinnamomum camphora*, *Euphorbia hirta*, *Azadirachta indica*, and *Carica papaya* plant extracts. Another plant, namely, *Camellia sinensis* (Abdel-Tawwab *et al*., 2010) was also proven to increase the growth performance and diet utilization index in Nile tilapia. This condition may occur related to the immuno-nutrient constituents, including the sugar complex like polysaccharides, which was believed to have prebiotic characteristics (Zahran *et al*., 2014) and could improve the nutrient digestibility, absorption, and animal assimilation capacity through the digestive tract morphological improvement (Heidarieh *et al*., 2013).

The lowest GSI value (D84, D98, and D112) was consistently obtained from the 1 g/kg diet dose treatment group, while the highest value was obtained from the control treatment (Table 4). These results were correlated to the histology results that indicate the gonadal inhibition (testis and ovary, Figure 3) compared to the control treatment group. The histological analysis in the 1 g/kg diet dose treatment group showed that the testis was still in the stage I in D84 & D98 with abnormal testis structure, namely, atrophy and degradation that caused the empty spermatogenic condition in many parts of testis. In D112 (stage II), a severe testis damage was occurred, namely, atrophy, degradation, and hydrophic degeneration; which indicates the greatest gonadal inhibition (Figure 3) compared to the control treatment group (Figure 1). The control treatment group fish histology showed that the gonad entered the stage III (D84 & D98) and stage IV (D112) with the normal structure and developmental phases (Kosai *et al*., 2011; Uribe *et al*., 2016). Similarly, the ovary histology in the 1 g/kg diet dose treatment group showed the ovary was in the stage I (D84) and stage II (D98 & D112), which indicates the occurrence of inhibition compared to the control treatment group that was in the stage III (D84), IV (D98), and V (D112). These results were similar to Jegede (2010) and Kushwaha (2013), that the *Hibiscus rosasinensis* extract at 2 g/kg diet and *Aloe vera* extract at 1 g/kg diet could degrade the testis and ovary of Nile tilapia, in addition to the *Carica papaya* extract at 2 g/kg diet could delay the Nile tilapia gonad maturation (Kareem *et al*., 2016). The inhibitory effect in the Nile tilapia fish was thought due to the bioactive compounds in the melastoma leaf extract, namely, cytosterol compounds (Farizah *et al*., 2017), which act as an inhibitor.

The cytosterol compounds can inhibit the aromatase and 5α-reductase enzymes (Rakel, 2018). These compounds have antagonistic characteristics against the estrogen receptor in the gonad nucleus that inhibits the steroid endogenous binding, mainly the estrogen hormone, which results in no response from the target cells (Manning, 2005l Retana-Marquez *et al*., 2012). Thereby, the steroidogenic enzyme inhibition and cellular estrogen receptor in Nile tilapia gonad will reduce the 17β-estradiol and prevent the testosterone conversion (Ramirez *et al*., 2017), which plays the role in the gonad development and maturation in fish (Guiguen *et al*., 2010). Consequently, the meat development in Nile tilapia occurred due to the energy was entirely used for the somatic growth, as obtained in the 1 g/kg diet dose treatment group with the highest growth performance.

These study results were also found in high dose (4 g/kg diet), as the inhibitory effect was only occurred in the ovary, namely in stage I (D84, Figure 5d), II (D98, Figure 5e), and III (D112, Figure 5f) compared to the control group treatment, which was in the stage V at the end of the maintenance period (Figure 1f). These results were similar to Farizah *et al*. (2017), who found that the inhibitory development of ovaries in the mud crab *Scylla olivacea* was

occurred in high dose. In contrast, testis remained inhibited with the histology results were in the stage II (D84, Figure 5a), III (D98, Figure 5b), and IV (D112, Figure 5c), which means that the developmental phase was relatively similar to the control treatment group (Figure 1a, b, c). The absent inhibitory effect in the Nile tilapia testis was thought due to the increased concentration of testosterone as the result of the continuous extract supplementation, which stimulated the testis maturity acceleration. This condition was observed from the high GSI value, mainly at the 4 g/kg diet dose treatment group (Table 4) and testis structure that returned to the normal condition during the maintenance period (Figure 5c, d). These results were supported by Farizah *et al.* (2017), who stated that the cytosterol compounds from the melastoma leaf extract could be used as a stimulator (aphrodisiac). However, this effect depends on each herbal extract; Abdelhak *et al.* (2013) reported a reversible characteristic after using the *Carica papaya* extract at 6 and 9 g/kg diet doses. Another impact observed was the decreased growth performance, mainly at the 4 g/kg diet dose treatment group (Table 3), which was thought due to the testis maturation acceleration that caused the slow growth (Omitoyin *et al.*, 2013), as the prospective energy for the somatic growth and biomass increase was used for gonad development (Kareen *et al.*, 2016; Kapinga *et al.*, 2018). Moreover, the decreased growth was also thought due to the high antinutrient concentrations (tannins and saponins), toxic constituents, exceeded dose, and allergic reaction factors (Yılmaz & Ergun, 2018).

CONCLUSION

The 1 g/kg diet dose treatment was the best dose to inhibit the gonad development of Nile tilapia and obtained the highest growth performance. The melastoma leaf extract can also potentially be utilized as a natural sex reversal agent in Nile tilapia.

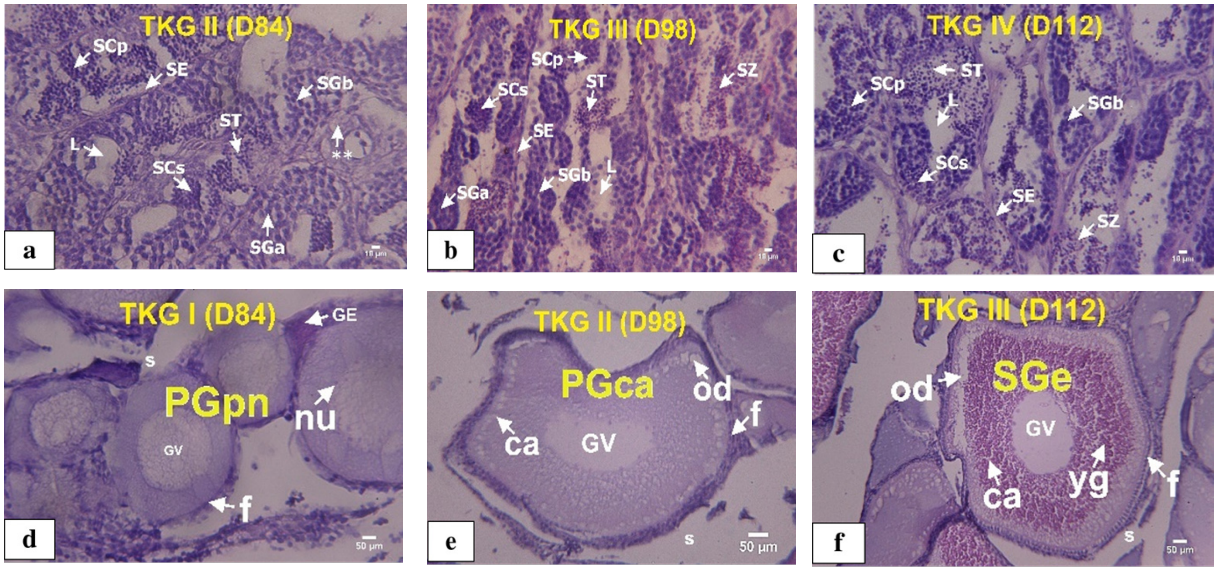

Figure 5. Testis (a, b, c) and ovary (d, e, f) histological results of Nile tilapia in the 4 g/kg diet treatment group. Note: a) testis histology (D84) in stage II dominated by the type B spermatogonia (SGb), primary spermatocytes (SCp), and less spermatids (ST) with testicule tubular atrophy & empty spermatogenic cyst; b) testis histology (D98) in stage III dominated by the secondary spermatocytes (SCs), spermatids (ST), and less spermatozoa (SZ); c) testis histology (D112) in stage IV dominated by the primary spermatocytes (SCp), secondary spermatocytes (SCs), and spermatids (ST), and more abundant to the spermatozoa (SZ) phase; d) ovary histology (D84) in stage I in the perinucleolar step of primary growth stage (PGpn); e) ovary histology (D98) in stage II in the cortical alveoli step of primary growth stage (PGca); f) testis histology (D112) in stage III in the early secondary growth step (SGe). Lumen (L), type A spermatogonia (SGa), Leydig cells (LE), Sertoli cells (SE), Stroma (S), nucleoli (nu), oil droplet (od)/lipid droplet, cortical alveoli (ca), germinal vesicle (GV), follicular cells (f), germinal epithelium (GE), **testicule tubular atrophy & empty spermatogenic cyst, and yolk globules (yg) (HE staining, bar scale: (a, b, c) 10 µm 100x magnification; (d, e, f) 50 µm 40x magnification).

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