Original Article

Decreasing duration of androgenic hormone feeding supplement for production of male monosex in tilapia (*Oreochromis* spp.) fry

Nion Vinarukwong[1] Mintra Lukkana[2] Janenuj Wongtavatchai[1*]

Abstract

The effects of an exogenous androgenic hormone on sex differentiation were examined in Nile tilapia (*Oreochromis niloticus*) and red tilapia (*Oreochromis* spp.) fry. Tilapia aquaculture in Thailand commonly applies methyltestosterone at the dose of 80 mg/kg diet on the first feeding of fish fry for 21-28 days to achieve male-monosex crop. In this study, male sex reversal was induced with an alternative androgenic hormone, 17α-methyldihydrotestosterone (MDHT), at a dose of 80 mg/kg diet for shorter periods; 5, 10, 15, or 20 days. Microscopic examination of fish gonad stained with aceto-carmine was used to determine male and female fish fry at 60 days post hatching (dph). Feeding supplementation with MDHT for up to 15 days yielded 100% male (n=100), while treatment for 5 and 10 days presented 87% and 90% male in Nile tilapia, and 82% and 88% in red tilapia, respectively. Histological examinations of the hormone-treated fish revealed normal microscopic morphology of tilapia gonadal tissues. In addition, analysis of total weight gain and body length among the fish fry showed no significant difference between the treatment groups and the control fish fed on basal diet. The present study demonstrated male sex reversal in tilapia fry with a decreased hormonal feeding to a 15-day period, while maintaining successful male-monosex and having no adverse effect on general fish growth.

Keywords: 17α-methyldihydrotestosterone, gonadal development, male-monosex, MDHT, tilapia fry

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Introduction

Tilapia is a species of major interest to aquaculturists worldwide due to its excellent growth rate, high yield, disease resistance, and tolerance to a wide range of environments. Tilapia is, thus, on its way to become a major supplier of protein in both the developed and developing world (FAO, 2016). The global production of farmed tilapia was 4,850,000 metric tons in 2014 and increased to 5,576,800 metric tons at the end of 2015 (Fitzsimmons, 2016). Sex control is desirable for aquaculture development to avoid unwanted spawning in a production unit. Male monosex is preferred in tilapia culture because males have a better feed conversion ratio and growth rate than the females (Singh, 2013).

There are various techniques that have been used to make male monosex for tilapia, including manual sexing, hybridization, genetic manipulation, environmental manipulation and hormonal sex reversal (Dauda et al., 2014). Manual sorting of sex is the simplest method requiring the least technology. However, this technique is laborious, needs to be applied by experienced, causes fish some stress and requires large size of fingerling to determine the sex (Cnaani and Levavi-Sivan, 2009). Hybridization is the crossing of two closely linked species but different subspecies of fish. If the sex determination system is different, the hybridization between a homogametic female and a homogametic male produces only male offspring (Wohlfarth and Wedekind, 1991; Bartley et al., 2000). The hybrids can increase growth rate, produce sterile progeny, increase disease resistance and improve tolerance to extreme environments (Al-Hakim et al., 2012; Mbiru et al., 2016). However, this method is not a perfect solution because of the difficulty in maintaining pure parental stock and the high costs associated with complicated equipment (Fortes, 2005). Genetic manipulation such as the induction of triploid fry, and the production of YY male genotypes with estrogen induced sex reversal of male to female brooder, leads to functional sterility that prevents unwanted reproduction in tilapia culture (Hussain et al., 1991; Mair et al., 1997; Pradeep et al., 2014). However, this method has been studied in laboratory scale, commercial application might be infeasible because of time and manpower consumed (Pradeep et al., 2012; Pradeep et al., 2014).

Environmental manipulation especially temperature alone can alter sex ratios of teleost fish (Phelps and Popma, 2000). The results of many studies show that temperature has an effect on sex differentiation during the developmental stage of embryo; high temperature makes fry become sex-incompatible females (Baroiller et al., 1999; Devlin and Nagahama, 2002; Wessels and Hörstgen-Schwark, 2007), but it is not a completely 100% success due to the differentiation into male or female in fish which is a complex and labile mechanism under the control of genetic, physiological and/or environmental factors (Devlin and Nagahama, 2002). Hormonal administration is the most commonly used chemical method for production of all-male population in farmed fish. There are several routes of hormonal administration including dietary feeding, immersion, injection, and implantation. Hormone injection and implantation are not practical in fish farming due to the cost, labour and skill requirement (Pandian and Sheela, 1995), whereas hormonal feeding and immersion are preferable techniques because of their reliability, high success rate, easy handling and cost effectiveness for farming practice (Haffray et al., 2009).

17α-methyldihydrotestosterone (MDHT) is the 17α-methylated version of dihydrotestosterone and suitable for clinical use because of its high anabolic potency without the undesirable masculinizing effects. It was introduced to be used for promoting nitrogen retention and weight gain in women with tuberculosis (Harris, 1961). MDHT was also used for prophylaxis and therapy of osteoporosis in postmenopausal woman issued in the United States Patent No. 5591735 (Mattern and Hacker, 1997). Since 1960s, MDHT has been one of the major doping substances used in athletes and racehorses for increasing muscle mass and physical strength (Franke and Berendonk, 1997; Fitch, 2008; Wong and Wan, 2014).

In this study the application of MDHT in Nile tilapia (*Oreochromis niloticus*) and red tilapia (*Oreochromis* spp.) fry was evaluated to define the proper dose for successful male sex reversal and adequate growth performance that is beneficial to tilapia production.

Materials and Methods

Hormonal preparation: MDHT (purity ≥98%) (Sigma-Aldrich, St Louis, Missouri, USA) was dissolved in 95% ethanol. The solution of MDHT for dietary supplementation was sprayed onto commercial fish feed. The moistened hormonal feeds were air dried and kept at 4°C under dark and dry conditions.

Animals: Nile tilapia and red tilapia obtained from hatchery unit of a commercial tilapia farm in Chachoengsao province were used for the experiment. Five thousand larvae of each Nile tilapia and red tilapia at the age of 10 dph were placed in hapas (1 m x 1.25 m x 0.8 m) at the stocking density of 250 fry/net. The water parameters were maintained as; mean temperature 29±3°C, pH 7.0-8.0, dissolved oxygen 7.0-8.0 mg/L, ammonia (NH_3) 0.00-0.50 mg/L, and nitrite 0.00-0.25 µg/L.

Hormonal treatment: Animal management was approved by the ethics committee of Chulalongkorn University Animal Care and Use Committee (CU-ACUC; Approval No. 11310046). Four groups (n=250 x 4 replications for each group) were fed 80 mg/kg MDHT with different durations (5, 10, 15 and 20 days) and the remaining groups were fed normal diet without MDHT to serve as control. Fish sexual differentiation and growth (body weight and length) to various dosages of MDHT dietary supplementation were determined at 60 days post hatching (dph) (Fig. 1).

Sexing: Sex percentages of each treatment (male and female) were determined from 25 fish of each

replication. Gonads of the fry were examined using gonad squash mount technique and stained with aceto-carmine for microscopic observation (Wassermann and Afonso, 2002). Histological development of the fish gonadal tissues was also investigated in fish aged 60, 80 dph and 10 months post hatching (mph).

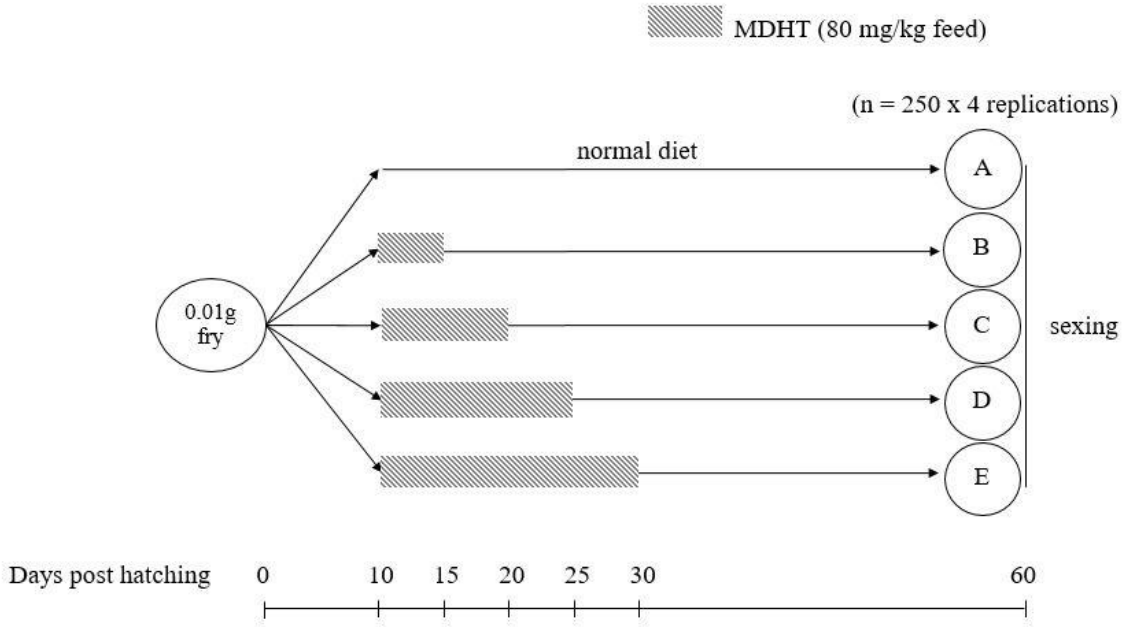

Figure 1 Schematic diagram showing 17α-methyldihydrotestosterone (MDHT) treatment in both Nile tilapia and red tilapia in groups A-E. A, control group; B to E, treated groups, MDHT feeding at 80 mg/kg diet for 5, 10, 15 and 20 days in groups B, C, D and E, respectively. After treatment, the fry were reared with a normal diet and fish gonad was examined at 60 days post hatching (dph).

Data analysis: Effects of the MDHT treatment on sex ratio, body weight and body length of fry were reported as mean±standard deviation (SD). Fulton's condition factor (Mortuza and Al-Misned, 2013) was also calculated using the equation, condition factor = $100W/L^3$, to assess the stoutness of the fish, where W is the body weight (g) and L is the body length (cm) of fish. Condition factor of each treatment was compared using one-way analysis of variance. Treatment effects were considered significant at $p < 0.05$ (SPSS statistical software version 22; SPSS Inc., Chicago, Illinois, USA).

Results

Using the fresh smear technique, the male gonadal tissue showed a tubular conformation; the female gonadal tissue showed a vesicle containing abundant round objects (Fig. 2). The feeding of MDHT at 80 mg/kg to 10 dph tilapia fry resulted in male/female ratio that deviated significantly from the normal 1:1 ratio (control group), with more male than female fish fry. The percent of phenotypic males increased with the longer duration of hormonal treatment, 87% male Nile tilapia and 82% male red tilapia in the 5-day treatment groups to 100% male Nile and red tilapia in the 15 and 20-day treatments (Tables 1-2). Histological development of the tilapia gonads examined at 60, 80 dph and 10 mph showed normal gonadal differentiation. Fig. 3 demonstrates histological sections of the gonads from MDHT-treated male at 60 dph, seminiferous tubules were observed in male gonads containing spermatogonia and primary spermatocytes with meiotic division. Spermatogonia increased rapidly in number, and spermatocytes developed, suggesting active spermatogenesis taking place in tilapia at the age of 80 dph. Mature testis showed testicular lobes with seminiferous tubules filled with germinal cells at different stages of development and surrounded by Sertoli cells. The spermatogenic cells were spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa (Fig. 3). Transverse and longitudinal sections of the female gonadal tissues showed round oocytes in various stages of oogenesis. Oogonia, and oocytes of perinucleolar stage were found during 60 dph. The perinucleolar stage was the oocyte with several nucleoli at the periphery of the nucleus. The layer of simple squamous cells surrounding each oocyte was follicular cells. The cortical alveolar stage of oocytes containing a germinal vesicle could be observed at 80 dph. At 10 mph, oocytes of different developmental stages were found in the mature ovary (Fig. 4).

In Nile tilapia, body weight, body length and condition factor were not different amongst the 4 treatment and control groups. Similar growth performance effect was observed in red tilapia (Tables 1-2).

Discussion

Many kinds of androgens have been used for male sex reversal, including administration of 11-ketotestosterone, 17α-methyltestosterone (MT), 5α-dihydrotestosterone (DHT) and MDHT (Leet et al., 2011). MT has been a hormone of choice for the production of all-male tilapia population worldwide

(Celik et al., 2011; Phelps and Okoko, 2011; Mateen and Ahmed, 2015) and the dosage of 60-80 mg MT/kg diet for 21-28 days of first feeding is applied in Thailand (Pongthana, 2010).

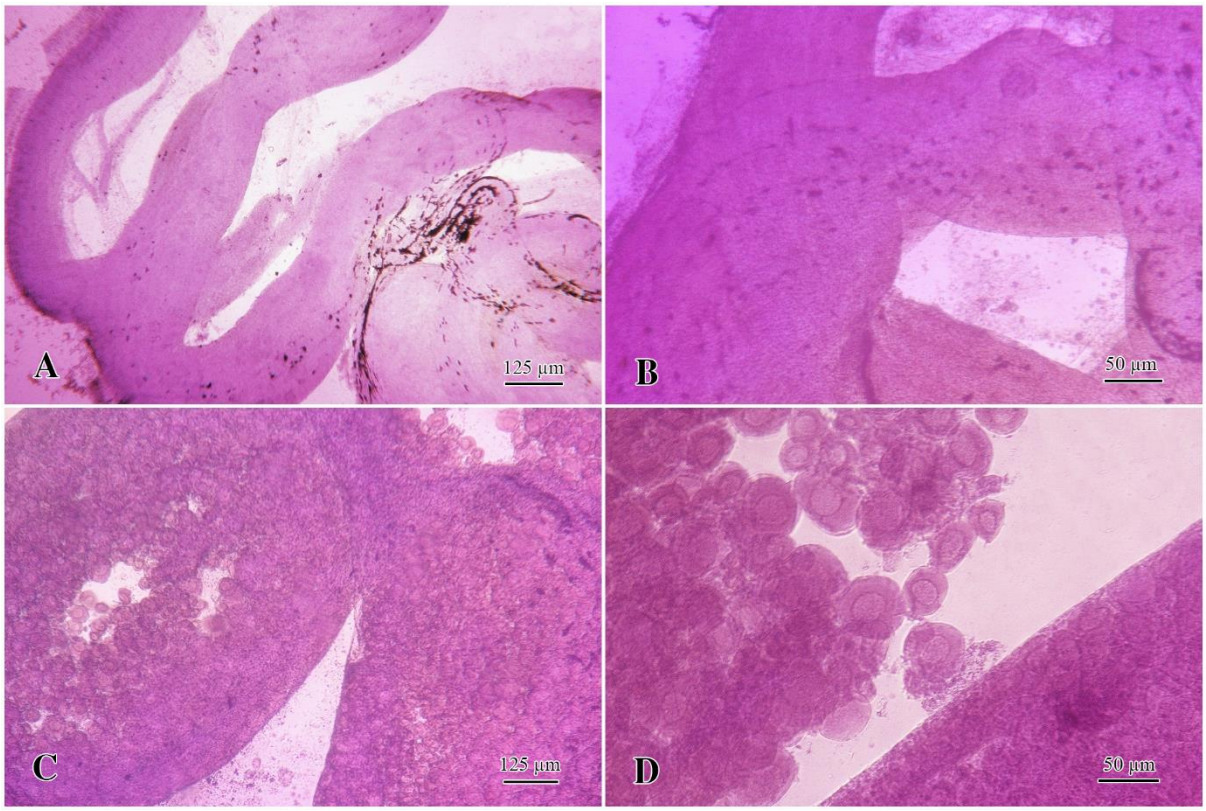

Figure 2 Gonadal tissues of 60 days post hatching (dph) tilapia stained with aceto-carmine. Male gonadal tissue showing a long smooth tube shape of the testis (A and B). Female gonadal tissue showing an ovary containing abundant round oocytes (C and D).

Table 1 Effect of MDHT on sex ratio and growth performance of Nile tilapia fry at age 60 days post hatching (dph) (mean±SD)

MDHT feeding (day)	Nile tilapia			
	male (%)	body weight (g)	body length (cm)	condition factor
0	53	12.82±0.66	8.51±0.45	2.12±0.35
5	87	12.79±0.76	8.49±0.39	2.11±0.29
10	90	12.85±0.70	8.51±0.33	2.10±0.26
15	100	12.78±0.73	8.45±0.46	2.15±0.33
20	100	12.75±0.84	8.44±0.44	2.15±0.32

MDHT, 17α-methyldihydrotestosterone; fed MDHT at 80 mg/kg feed (n=100 of each treatment)

Table 2 Effect of MDHT on sex ratio and growth performance of red tilapia fry at age 60 days post hatching (dph) (mean±SD)

MDHT feeding (day)	Red tilapia			
	male (%)	body weight (g)	body length (cm)	condition factor
0	48	11.73±0.77	7.75±0.35	2.54±0.37
5	82	11.78±0.84	7.84±0.31	2.46±0.30
10	88	11.75±0.76	7.83±0.39	2.47±0.34
15	100	11.79±0.78	7.81±0.40	2.51±0.35
20	100	11.65±0.83	7.82±0.40	2.47±0.36

MDHT, 17α-methyldihydrotestosterone; fed MDHT at 80 mg/kg feed (n=100 of each treatment)

According to the results of this study, MDHT is an alternative androgenic hormone to produce all-male tilapia within less than 21-18 days MT feeding. The body weight, body length and condition factor of fry were recorded only one time at the day of sexing (60 dph) and no significant differences between the MDHT-treated groups and the control group (p >0.05) were found. In addition, condition factor values determined in all groups were >1, suggesting effective growth in fish species (Malik et al., 2017). This implies that the MDHT treatment did not affect the growth rate. The histological examinations revealed no differences between the gonadal tissues treated with exogenous MDHT and those of the non-treated fish. The differentiation of male gonad observed in this study closely matches earlier studies in Nile tilapia treated with MT feeding supplements (Esterhuyse et al., 2008; El-Sakhawy et al., 2011; El-Saba et al., 2013).

The effect of exogenous hormones on sex differentiation in Nile tilapia was explained with the gene expression mechanism. The expression of *cyp19a* (a gene producing aromatase enzyme responsible for ovarian differentiation and the conversion of androgens into 17β-estradiol) starts at 5 dph in

genotypic female while the expression of *dmrt1* (a gene involving testicular differentiation) displays at 6 dph in genotypic male. The study indicated that the differential expression of these genes during the period of 5-6 dph is critical for differentiation of the gonads to ovary or testis (Ijiri et al., 2008). The oral administration of exogenous androgen increases dmrt1, which plays a role as a transcription suppressor of *cyp19a*, resulting in interrupted ovarian differentiation, then the male sex imposes (Bhandari et al., 2006; Piferrer, 2011). In addition, exogenous androgen induces sex reversal in genotypic female *via* androgen receptors (*ar1* and *ar2*) detectable in female gonads at 5 dph and causes genotypic female fry responsive to masculinization (Tao et al., 2013). The sensitive period for gonadal development described in earlier studies suggest the most precisely timed and thus effective androgenic treatments in Nile tilapia at 5-6 dph (Ijiri et al., 2008).

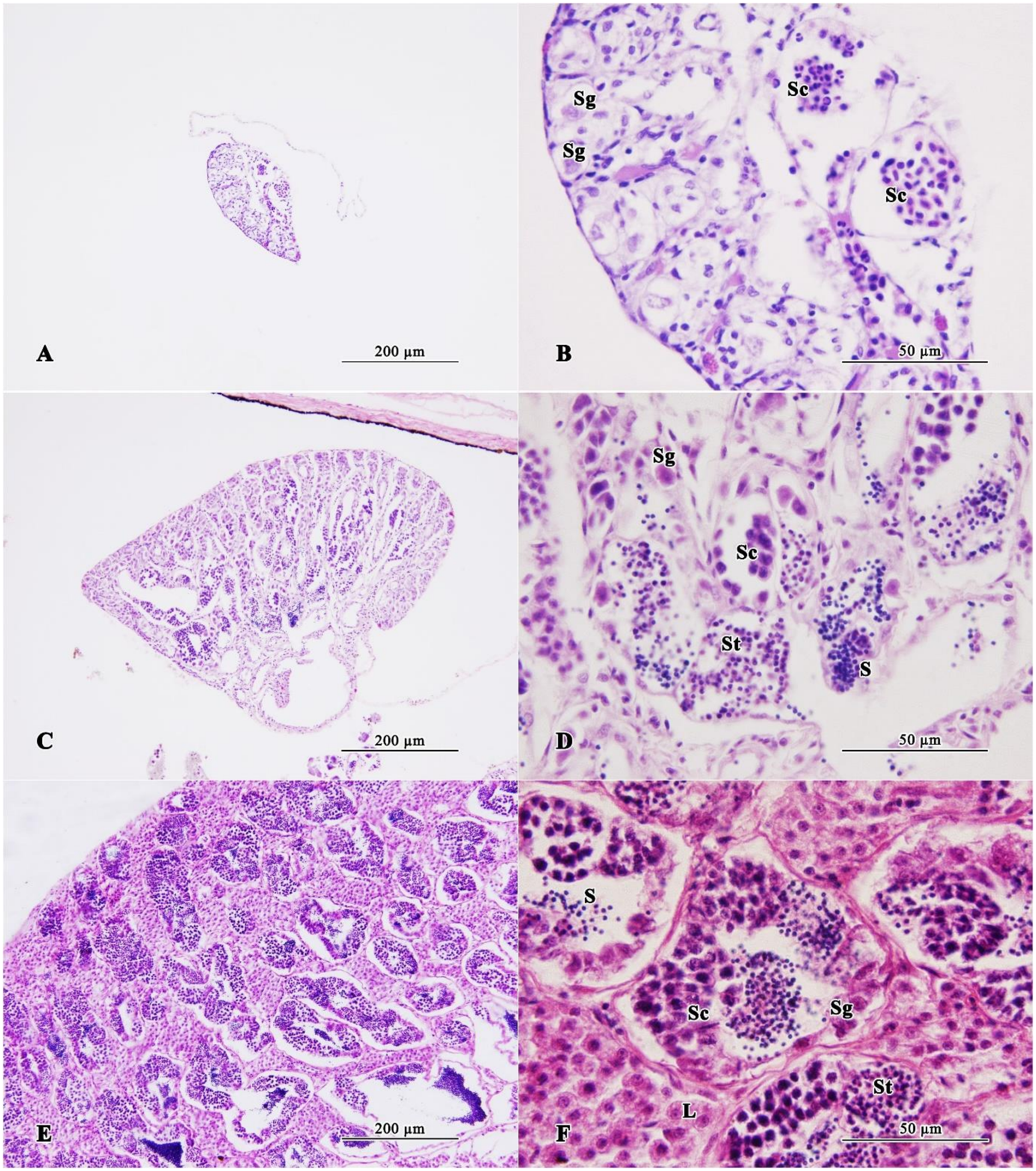

Figure 3 Histology of the testicular development of tilapia with H&E staining. Transverse sections of the male gonadal tissue showing a bean shaped testis and seminiferous tubules at 60 days post hatching (dph) (A and B). Active spermatogenesis in the testis at 80 dph (C and D). Many cysts containing spermatogenic germ cells at various stages were distributed throughout the testis, including spermatogonia (Sg), spermatocytes (Sc), spermatids (St) and spermatozoa (S). Mature testis with abundant Leydig cells (L) at 10 months post hatching (mph) was shown (E and F).

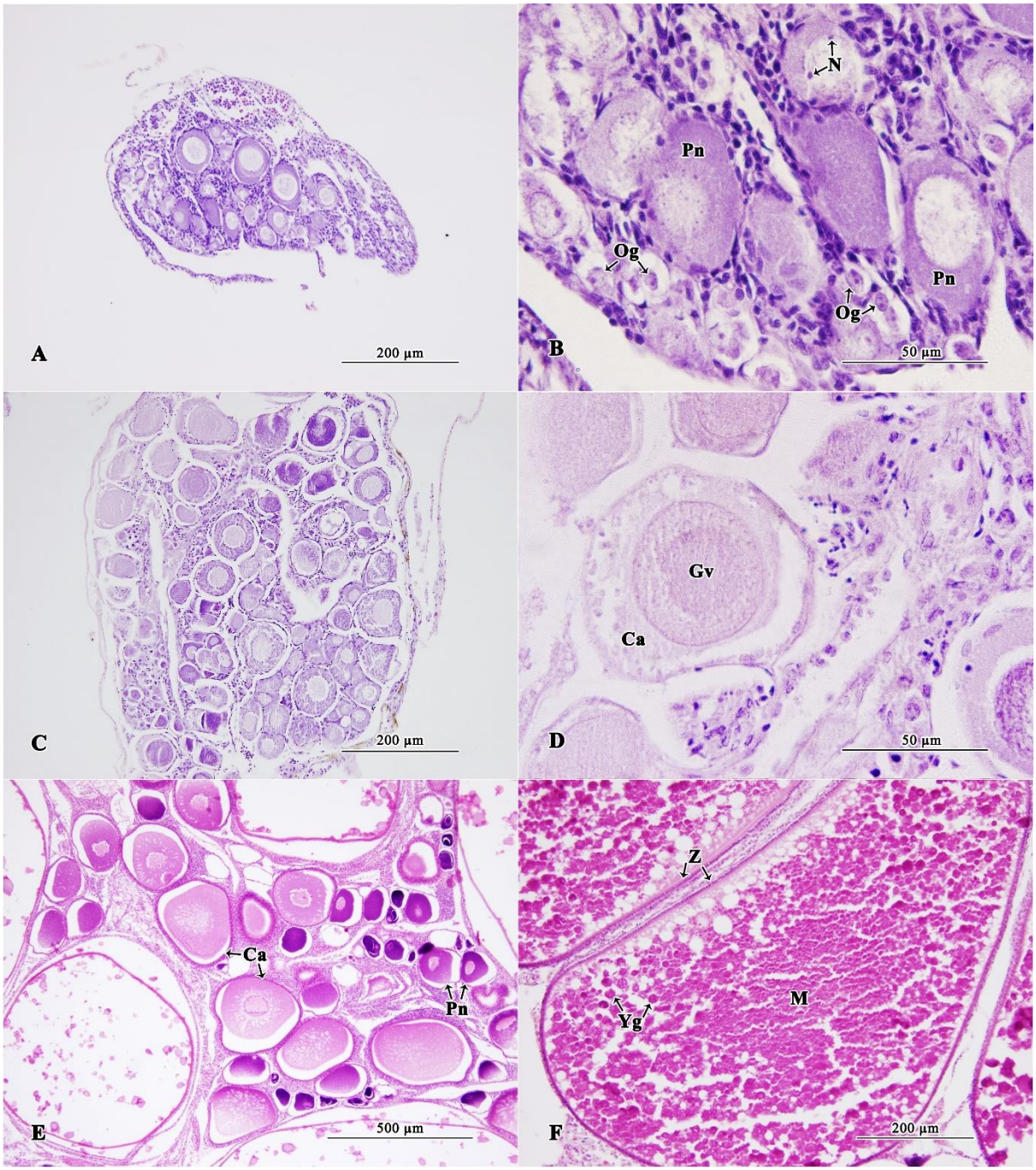

Figure 4 Histology of the ovaries development of tilapia with H&E staining. Transverse sections of 60 days post hatching (dph) female gonadal tissue showing oogonia (Og) and perinucleolar stage of oocytes (Pn) with several small nucleoli (N) attached to the nuclear membrane (A and B). The cortical alveolar stage of oocytes (Ca) containing a germinal vesicle (Gv) could be observed at 80 dph (C and D). At 10 mph, abundant oocytes in various stages embedded in ovarian interstitial tissue. Mature follicles (M) contained cytoplasm which is full of large yolk globules (Yg) and surrounded by trilayers consisting of acidophilic zona pellucida (Z), cuboidal follicular cell layer and stratified squamous thecal cell layer (E and F).

The potential problem encountered when treating with high dose or long-term MT is paradoxical feminization as shown in Nile tilapia. El-Greisy and El-Gamal (2012) found an increasing frequency of males up to a dose of 60 mg/kg diet (97.0% male), whereas the percentage of male declined to 93.0% at the higher dose, 80 mg/kg diet. Mateen and Ahmed (2007) reported a similar result obtaining 95.4% male with 70 mg MT/kg diet, but 80 mg MT/kg diet yielded only 90.7% male. This phenomenon might be associated with the aromatization which converses MT to estrogen and consequently promotes ovarian differentiation (Piferrer and Donaldson, 1991; Piferrer et al., 1994). In contrast to the aromatizable MT, MDHT is a methylated form of DHT, and the 5 α-reduction prevents aromatization, so called non-aromatizable MDHT (Li et al., 2006). Thus, the more effectiveness of MDHT than MT might be due to partial aromatization of MT to estradiol. Our study agrees with a previous study by Piferrer et al. (1993) showing that MDHT was twice potent as MT in masculinizing female chinook salmon (*Oncorhynchus tshawytscha*); this might be the reason why we could use the less dosage of MDHT (15 days of administration) in this study.

In summary, the present study shows that a shortened duration of MDHT feeding can produce male monosex tilapia, thus, increasing management effectiveness for the aquaculturist. More importantly, the less hormonal use in aquaculture, the less hormonal residue depositing in fish products and environment. Nevertheless, further study to determine the hormonal depletion in hormone-treated fish would ensure that the treated fish is safe for consumption at the marketable size.

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References

Al-Hakim NFA, Saleh M, Hegazi AZ, Ibrahim A, Aly K and Tahoun MA 2012. Induction of mono-sex (male tilapia) population by inter-specific hybridization and hormonal sex reversal of Nile tilapia. Egypt J Aquat Biol Fish 1: 23-33.

Baroiller JF, Guiguen Y and Fostier A 1999. Endocrine and environmental aspects of sex differentiation in fish. Cell Mol Life Sci. 55(6): 910-931.

Bartley D, Rana K and Immink A 2000. The use of inter-specific hybrids in aquaculture and fisheries. Rev Fish Biol Fish. 10(3): 325-337.

Bhandari RK, Nakamura M, Kobayashi T and Nagahama Y 2006. Suppression of steroidogenic enzyme expression during androgen-induced sex reversal in Nile tilapia (*Oreochromis niloticus*). Gen Comp Endocrinol. 145(1): 20-24.

Celik I, Guner Y and Celik P 2011. Effect of orally-administere d 17α-methyltestosterone at different doses on the sex reversal of the Nile tilapia (*Oreochromis niloticus*, Linneaus 1758). J Anim Vet Adv. 10(7): 853-857.

Cnaani A and Levavi-Sivan B 2009. Sexual development in fish, practical applications for aquaculture. Sex Dev. 3(2-3): 164-175.

Dauda AB, Yakuba SO and Oke AO 2014. Curbing the menace of prolific breeding in "aquatic chicken" (tilapia): A way out to improve fish production in Nigeria. N Y Sci J. 7(4): 112-118.

Devlin RH and Nagahama Y 2002. Sex determination and sex differentiation in fish: an overview of genetic, physiological, and environmental influences. Aquaculture. 208(3-4): 191-364.

El-Greisy ZA and El-Gamal AE 2012. Monosex production of tilapia, *Oreochromis niloticus* using different doses of 17α-methyltestosterone with respect to the degree of sex stability after one year of treatment. Egypt J Aquat Res. 38(1): 59-66.

El-Saba A, Abd Rabou M, El-Sakhawy M, El-Shammaa M and Hussein S 2013. Seasonal changes in the histology of the ovaries of Nile tilapia (*Oreochromis niloticus*). J Vet Anat 6(2): 1-21.

El-Sakhawy M, El-Saba A, Abd Rabou M, El-Shammaa M and Hussein S 2011. Seasonal histology of the testes of Nile tilapia (*Oreochromis niloticus*). J Vet Anat 4(2): 61-75.

Esterhuyse M, Helbing C and Van Wyk J 2008. Temporal expression of two Cytochrome P450 Aromatase isoforms during development in *Oreochromis mossambicus*, in association with histological development. Comp Biochem Physiol D Genom Proteomics. 3(4): 297-306.

FAO 2016. "The State of World Fisheries and Aquaculture 2016". [Online]. Available: http://www.fao.org/3/a-i5555e.pdf.

Fitch KD 2008. Androgenic-anabolic steroids and the Olympic Games. Asian J Androl. 10(3): 384-390.

Fitzsimmons K 2016. "Supply and demand in global tilapia markets". [Online]. Available: https://www.was.org/meetings/ShowAbstract.aspx?Id=41768.

Fortes R 2005. Review of techniques and practices in controlling tilapia populations and identification of methods that may have practical applications in Nauru including a national tilapia plan. Aquaculture Technical Paper/Secretariat of the Pacific Community. 55.

Franke WW and Berendonk B 1997. Hormonal doping and androgenization of athletes: a secret program of the German democratic republic government. Clin Chem. 43(7): 1262-1279.

Haffray P, Petit V, Guiguen Y, Quillet E, Rault P and Fostier A 2009. Successful production of monosex female brook trout *Salvelinus fontinalis* using gynogenetic sex reversed males by a combination of methyltestosterone immersion and oral treatments. Aquaculture. 290(1-2): 47-52.

Harris L 1961. The protein anabolic action of mestanolone. J Clin Endocrinol Metab. 21(9): 1099-1105.

Hussain MG, Chatterji A, McAndrew B and Johnstone R 1991. Triploidy induction in Nile tilapia, *Oreochromis niloticus* L. using pressure, heat and cold shocks. Theor Appl Genet. 81(1): 6-12.

Ijiri S, Kaneko H, Kobayashi T, Wang DS, Sakai F, Paul-Prasanth B, Nakamura M and Nagahama Y 2008. Sexual dimorphic expression of genes in gonads during early differentiation of a teleost fish, the Nile tilapia *Oreochromis niloticus*. Biol Reprod. 78(2): 333-341.

Leet JK, Gall HE and Sepúlveda MS 2011. A review of studies on androgen and estrogen exposure in fish early life stages: effects on gene and hormonal control of sexual differentiation. J Appl Toxicol. 31(5): 379-398.

Li GL, Liu XC and Lin HR 2006. Effects of aromatizable and nonaromatizable androgens on the sex inversion of red-spotted grouper (*Epinephelus akaara*). Fish Physiol Biochem. 32(1): 25-33.

Mair G, Abucay J, Abella T, Beardmore J and Skibinski D 1997. Genetic manipulation of sex ratio for the large-scale production of all-male tilapia *Oreochromis niloticus*. Can J Fish Aquat Sci. 54(2): 396-404.

Malik A, Abbas G, Smmmro MA, Shah SSA, Asadullah A, Bhutto AH, Jamali GQ and Roonjho Z 2017. Length-weight relationship and condition factor of red tilapia (hybrid) reared in cemented tanks of sun-bright red tilapia and ornamental hatchery-karachi, Sindh-Pakistan. Sindh Univ Res Jour. (Sci Ser.) 49(1): 159-162.

Mateen A and Ahmed I 2007. Effect of androgen on sex reversal and growth of Nile tilapia (*Oreochromis niloticus*). Pak J Agri Sci. 44(2): 272-276.

Mateen A and Ahmed I 2015. Androgen sex reversal, subsequent growth and meat quality of Nile tilapia (*Oreochromis niloticus*). Pak J Agr Sci. 52(1): 199-202.

Mattern C and Hacker R 1997. Medicament for influencing the degree of activation of the central nervous system. U.S. Patent No. 5,591,732. Washington, DC: U.S. Patent and Trademark Office.

Mbiru M, Limbu SM, Chenyambuga SW, Lamtane HA, Tamatamah R, Madalla NA and Mwandya AW 2016. Comparative performance of mixed-sex and hormonal-sex-reversed Nile tilapia *Oreochromis niloticus* and hybrids (*Oreochromis niloticus*× *Oreochromis urolepis hornorum*) cultured in concrete tanks. Aquacul Int. 24(2): 557-566.

Mortuza MG and Al-Misned FA 2013. Length-weight relationships, condition factor and sex-ratio of Nile tilapia, *Oreochromis niloticus* in Wadi Hanifah, Riyadh, Saudi Arabia. World J Zool. 8(1): 106-109.

Pandian TJ and Sheela SG 1995. Hormonal induction of sex reversal in fish. Aquaculture. 138(1-4): 1-22.

Phelps RP and Okoko M 2011. A non-paradoxical dose response to 17α-methyltestosterone by Nile tilapia *Oreochromis niloticus* (L.): effects on the sex ratio, growth and gonadal development. Aquacult Res. 42(4): 549-558.

Phelps RP and Popma TJ 2000. Sex reversal of tilapia. Tilapia Aquacult in Am. 2: 34-59.

Piferrer F 2011. Hormonal control of reproduction and growth: Endocrine control of sex differentiation in fish. In: Encyclopedia of Fish Physiology. ed. Editor-in-Chief: Anthony P. Farrell (ed.). San Diego: Academic Press. p. 1490-1499.

Piferrer F, Baker IJ and Donaldson EM 1993. Effects of natural, synthetic, aromatizable, and nonaromatizable androgens in inducing male sex differentiation in genotypic female chinook salmon (*Oncorhynchus tshawytscha*). Gen Comp Endocrinol. 91(1): 59-65.

Piferrer F and Donaldson EM 1991. Dosage-dependent differences in the effect of aromatizable and nonaromatizable androgens on the resulting phenotype of coho salmon (*Oncorhynchus kisutch*). Fish Physiol Biochem. 9(2): 145-150.

Piferrer F, Zanuy S, Carrillo M, Solar II, Devlin RH and Donaldson EM 1994. Brief treatment with an aromatase inhibitor during sex differentiation causes chromosomally female salmon to develop as normal, functional males. J Exp Zool A Ecol Genet Physiol. 270(3): 255-262.

Pongthana N 2010. Extension paper No. 2/2010. Department of Fisheries. Ministry of Agriculture and Cooperatives. Bangkok, Thailand. 47 p.

Pradeep PJ, Srijaya TC, Hassan A, Chatterji AK, Withyachumnarnkul B and Jeffs A 2014. Optimal conditions for cold-shock induction of triploidy in red tilapia. Aquac Int. 22(3): 1163-1174.

Pradeep PJ, Srijaya TC, Papini A and Chatterji AK 2012. Effects of triploidy induction on growth and masculinization of red tilapia [*Oreochromis mossambicus* (Peters, 1852)× *Oreochromis niloticus* (Linnaeus, 1758)]. Aquaculture. 344-349: 181-187.

Singh AK 2013. Introduction of modern endocrine techniques for the production of monosex population of fishes. Gen Comp Endocr. 181: 146-155.

Tao W, Yuan J, Zhou L, Sun L, Sun Y, Yang S, Li M, Zeng S, Huang B and Wang D 2013. Characterization of gonadal transcriptomes from Nile tilapia (*Oreochromis niloticus*) reveals differentially expressed genes. PLoS One. 8(5): e63604.

Wassermann GJ and Afonso LOB 2002. Validation of the aceto-carmine technique for evaluating phenotypic sex in Nile tilapia (*Oreochromis niloticus*) fry. Cienc Rural. 32(1): 133-139.

Wessels S and Hörstgen-Schwark G 2007. Selection experiments to increase the proportion of males in Nile tilapia (*Oreochromis niloticus*) by means of temperature treatment. Aquaculture. 272, Supplement 1(0): S80-S87.

Wohlfarth GW and Wedekind H 1991. The heredity of sex determination in tilapias. Aquaculture. 92: 143-156.

Wong JK and Wan TS 2014. Doping control analyses in horseracing: a clinician's guide. Vet J. 200(1): 8-16.

บทคัดย่อ

การลดระยะเวลาให้ฮอร์โมนเพศผู้ผสมอาหารเพื่อการผลิตลูกปลานิลเพศผู้

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การเพาะเลี้ยงปลานิลของประเทศไทยใช้ฮอร์โมนเมทิลเทสโทสเตอโรนขนาด 80 มิลลิกรัมต่อกิโลกรัมอาหาร ให้ลูกปลากินตั้งแต่วันแรกที่กินอาหาร เป็นระยะเวลาติดต่อกัน 21-28 วัน เพื่อเหนี่ยวนำให้เป็นเพศผู้ การศึกษานี้ทดสอบผลของฮอร์โมนแอนโดรเจนอีกชนิดหนึ่งต่อพัฒนาการทางเพศในลูกปลานิล (*Oreochromis niloticus*) และลูกปลานิลแดง (*Oreochromis* spp.) ด้วยการใช้ฮอร์โมน 17 แอลฟาเมทิลไดไฮโดรเทสโทสเตอโรน (MDHT) ขนาด 80 มิลลิกรัมต่อกิโลกรัมอาหาร ให้ลูกปลากินตั้งแต่วันแรกที่กินอาหารติดต่อกัน 5, 10, 15 หรือ 20 วัน และตรวจเพศของลูกปลาอายุ 60 วันหลังฟักเป็นตัวด้วยการใช้กล้องจุลทรรศน์ตรวจลักษณะของอวัยวะสืบพันธุ์ที่ย้อมสีอะซีโตคามีน การศึกษาพบว่าลูกปลานิลและปลานิลแดงที่ได้รับ MDHT ผสมอาหารเป็นเวลา 15 หรือ 20 วันเป็นเพศผู้ 100% (n=100) ลูกปลาที่ได้รับฮอร์โมนผสมอาหาร 5 และ 10 วันเป็นเพศผู้ 87% และ 90% ในปลานิล และ 82% และ 88% ในปลานิลแดง ตามลำดับ ลักษณะทางจุลกายวิภาคของเนื้อเยื่ออวัยวะสืบพันธุ์ในลูกปลากลุ่มที่ได้รับฮอร์โมนผสมอาหารแสดงลักษณะปกติ ลูกปลาที่ได้รับฮอร์โมนผสมอาหารมีอัตราการเจริญเติบโตประเมินจากน้ำหนักและความยาวลำตัวไม่แตกต่างจากกลุ่มควบคุม การศึกษานี้แสดงให้เห็นการเหนี่ยวนำลักษณะเพศผู้ในลูกปลานิลด้วยการให้ MDHT ผสมอาหารเพียง 15 วัน โดยไม่มีผลเสียต่อการเจริญเติบโตของลูกปลา

คำสำคัญ: 17 แอลฟาเมทิลไดไฮโดรเทสโทสเตอโรน พัฒนาการของอวัยวะสืบพันธุ์ เพศผู้เพศเดียว เอ็มดีเอชที ลูกปลานิล

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