E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2018; 6(3): 301-305

www.fisheriesjournal.com
Received: 09-03-2018
Accepted: 10-04-2018

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Orally administered 17 α methyl testosterone at different doses on the sex reversal of the red tilapia (*Oreochromis niloticus*)

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Abstract
All male population, the red tilapia larvae which just finished the yolk sac were fed with feed containing 17 α Methyltestosterone at five different doses (0, 30, 40, 50 and 60 mgMT/kg feed) for 30 days, After 30 days, the fry were shifted to 15 cemented cisterns continued further for 4 months with commercial (Basal) feed for further growth and checking the sex ratio after sexual maturity under different treatments. It was found out that the sex reversal rates among the groups were at the levels of 40.0, 60.0, 76.67, 100.0 and 100.0%, respectively. Beside the highest male population in hormone treated fish, the growth of the fish was also higher in treatments than control. The oral application of hormone significantly increased male population as compared to control. The highest sex reversal rate was observed in the group to which 50 mgMT/kg feed dose was applied.

Keywords: Sex reversal, 17- α methyl testosterone, red tilapia, growth

Introduction
Tilapia is the generic name of a group of cichlids endemic to Africa. The group consists of three aquaculturally important genera - *Oreochromis*, *Sarotherodon* and *Tilapia*. Several characteristics distinguish these three genera, but possibly the most critical relates to reproductive behavior. All *tilapia* species are nest builders; fertilized eggs are guarded in the nest by a brood parent. Species of both *Sarotherodon* and *Oreochromis* are mouth brooders; eggs are fertilized in the nest but parents immediately pick up the eggs in their mouths and hold them through incubation and for several days after hatching. In *Oreochromis* species only females practice mouth brooding, while in *Sarotherodon* species either the male or both male and female are mouth brooders. To overcome the negative aspects such as prolific breeding habit and slower growth rate of female, tilapia culture has traditionally involved all-male populations. The most common method for producing mono sex populations (all-male) is the sex reversal of larvae with the use of feeds containing synthetic sex hormones, 17*α*-methyltestosterone (MT). Males of tilapia usually grow faster than females [1, 2]. Culture of all male tilapia is preferred, because it takes advantage of fast growth rate and eliminating reproduction at the same time [3]. Dosage of 17α- methyl testosterone (MT) used to produce all male tilapia vary widely. The dosage rates vary from 10-70 mg hormone/Kg of diet for Nile tilapia, *Oreochromis niloticus*. The duration of administrating 17α- methyl testosterone (MT) for masculinization of Nile tilapia varies from 14 to 60 days [4-6]. Oral administration of 17α-MT at 40-60 mg.kg^{-1} feed from first feeding for a duration of 3-4 week produced about 98-100% male tilapia. If the fry are cultured in green water where plenty of natural food is available, then 60 mg MT.kg^{-1} diet is required [7].

Materials and Methods
Experimental Fish: For conducting this study, the fish "Red Tilapia" was choosen as experiment fish. The fish belongs to family Cichlidae, which is basically originated from Africa. The original red tilapia is genetic mutants. The first red tilapia was produced in Taiwan in the late 1960s, by a cross between a mutant reddish-orange female Mozambique tilapia and a normal male Nile tilapia.

Preparation of Basal Diet
A basal diet was prepared using 30% fish meal, 25% groundnut oil cake, 22% rice bran, 15% yellow corn, 6% corn oil and 2% vitamins and minerals premix. All the ingredients were thoroughly mixed and ground to prepare fine powder.

Hormone Diet Preparation and Feeding Phase
The experimental diet was prepared by mixing hormone in basal diet. For this purpose five quantities of MT 0 (C), 30 (T_1), 40 (T_2), 50 (T_3) and 60 mg (T_4) were weighed and dissolved separately in ethanol. The ethanol dissolved hormone was sprayed over (one kg for each dose) basal diet and mixed well and dried for 2 hours under shed. Thus the hormone mixed diets were finally converted to small pellets (<2.0mm) and kept in refrigerated for further use. The hormone feeding phase was continued for a period of 30 days. The fish were then fed with hormone free commercial (Basal) diet for further four months.

Growth Parameters: The mean length, weight and survival of the fish in each treatment were recorded. After 150 days post-hatching, at which the fish sexually mature completely, the percentage of males and females was recorded.
Water quality parameter *viz*., temperature, pH, dissolved oxygen, EC, total dissolved solids and nitrate-nitrogen in experimental systems were analyzed following standard methods [8].

Sexing of Hormone Treated Fish
A total of ten per cent fish samples were randomly obtained from each replication after 5 months for gonad examination. The sex of hormone treated tilapia was examined on the basis of genital papilla. The sex of the fish was initially determined by the external examination of the genital papilla and then verified later by the dissection of the gonads. The distinctive features of the genital papilla of the male and female tilapia [9]. The male has two orifices situated just forward of the anal fin. One is anus and the other is urogenital aperture which usually forms into a small papilla. The female, in contrast, has three orifices, namely the anus, a transverse genital opening and a microscopic urinary orifice which is scarcely visible to the naked eye.

Results
The hormone (17α-methyl testosterone) treated diet (0, 30, 40, 50 and 60 mg/kg diet) was fed to the fry of red tilapia for a total period of 30 days. After 30 days, the fry were shifted to 15 cemented cisterns for further growth and checking the sex ratio after sexual maturity under different treatments.

Growth Parameters
The sex ratios of red tilapia fed with hormone treated diets are shown in Table 1. The control showed a normal sex ratio, with 40% male and 60% female, while all other treatments received MT showed a greater and significant higher male proportion as compared to the control. The female proportion also showed significant differences among control and MT treatments. In both T4 (60 mg $MT.kg^{-1}$) and T3 (50 mg $MT.kg^{-1}$), the male population was 100 percent. The treatments T_2 (40 mg $MT.kg^{-1}$), T1 (30 mg MT.kg-^{1}) and Control (0 mg $MT.kg^{-1}$) have male population of 76.67, 60.00 and 40.00% respectively. Further, the female proportion was 60.00, 40.00, 23.33, 0.00 and 0.00% in control (0 mg $MT.kg^{-1}$), T_1 (30 mg $MT.kg^{-1}$), T_2 (40 mg $MT.kg^{-1}$), T_3 (50 mg $MT.kg^{-1}$) and T_4 (60 mg $MT.kg^{-1}$), respectively. The results of this study clearly depicted the impact of hormone treatment on male production. The highest impact was seen in T_3 and T_4 with 100% male populations (Table 1). The data pertaining to per cent change in male and female populations in treatments over control are presented in Fig 1. It is evident from this figure that the male populations positively increased with increasing hormone dose. On the other hand, female population decreased with increasing dose of hormone (Fig. 1).
Statistical analysis on sex reversal data showed highly significant difference (P<0.01) among the sexes (i.e. male and female) for all the treatments as compared to control. The mean values of male and female populations showed a significant difference as compared to control. However, both male and female populations were not statistically different in T_3 and T_4 (Table1).

Growth Parameters: After feeding on hormone treated diets for 30 days the fish were transferred to cemented cisterns of 3x3x1m size for growth studies. The fish were fed on formulated feed (Growell) @ 10% of body weight daily. The growth parameters (i.e. weight gain and total length, per cent weight gain, SGR etc. were recorded fortnightly. The growth data obtained are presented in Table 2. The hormone application rates significantly affected the growth rate. In all the treatments fish growth was higher than control. Still, the highest (249.81g) growth was noticed in T_4 followed by T_3 (50 mg MT), T_2 (40 mg MT), T_1 (30 mg MT) and control (0 mg MT) with respective weight gain in of 197.18, 175.02, 133.72 and 113.91 g (Table 2). The statistical analysis of the results (ANOVA) on net weight gain indicated significant results (P<0.05). Further, the critical difference (CD = 16.824) has also shown significant differences between treatments and control. The maximum SGR values of 4.48 per cent was observed in T_4 while minimum of 3.96 per cent was observed in control followed by T_3, T_2 and T_1 with respective values of 4.33, 4.25 and 4.07%. The statistical analysis (ANOVA) indicated a significant (P<0.05) impact of hormone treatment on SGR. Similarly, the critical difference (CD = 0.080) has also indicated significant difference among different treatments and control.
The average total length and gain in total length had a marked differences among different treatments. All the treatments showed higher gain in total length than control (Table 2). Still the highest (14.35cm) length gain was obtained in T_4 (60 mg MT) followed by treatments T_3 (50 mg MT), T_2 (40 mg MT) and T_1 (30 mg MT) with respective length gains of 14.34, 14.25 and 14.23 cm. The lowest was observed in control (14.19 cm). The statistical analysis (ANOVA) for total length gain indicated a significant (P<0.05) difference between treatments and control. The critical difference (CD = 0.256) also indicated significant difference among treatments and control. However, there was no significant difference within treatments.

Survival: The survival (%) of experimental fish is presented in fig. 2. That the treatment T_4 (60 mg MT) had maximum survival (77.33%), while in control (0 mg MT) the survival was only 61.33%. The survival in treatment T_3 (50 mg MT), T_2 (40 mg MT) and T_1 (30 mg MT) were 70.7, 69.33 and 68.0% respectively. The statistical analysis (ANOVA) on survival indicated a significant (P<0.05) impact of hormone treatment. The critical difference (CD = 0.75153) has also

indicated significant difference between treatments and control. However, there was no significant difference among the treatments.

Physico-chemical Parameters of Water

The water temperature ranged between a minimum of 19.35 ºC and maximum of 28.65 ºC. The trend in pH fluctuation remained similar in all treated tank throughout the experimental duration. It fluctuated between 7.1 to 8.42 with minimum and maximum respectively. The highest (2.06 mMho/cm^2) and lowest (1.97 mMho/cm^2) mean values were also found in treated tank and untreated (control) tank. Highest and lowest (8.06 mg/l) and (5.25 mg/l) value was observed in treated tank and TDS value were highest in treated tank compare to control and Nitrate-nitrogen was recorded in 0.045 mg/l in untreated (control) tank.

Discussion

This study demonstrates that the oral administration of 17α-methyl testosterone at 0, 30, 40, 50 and 60 mg MT/kg diet has significant potential in sex reversal from female to male in red tilapia. In this study, the hormone mixed feed was given at 10% of body weight daily which resulted in up to a 100% male population with 50 and 60 mg MT/kg doses. Obtained 98% male population at the dose of 60 mg MT/kg of feed [10]. Reported 99-100% male Nile tilapia when given MT at 60 mg/kg of feed. However, a significantly lower male proportion (91.43%) for highest dose (70 mg MT/kg of feed) was reported [11]. Similar result was found, at the dose of 30mg MT/kg maximum 99.3% males and at the dose of 60 and 120 mg MT/kg minimum 97 and 71.9% males [12]. He further reported that higher dose rates of MT/kg of feed resulted in no increase of male percentage.

The objective of this study was to determine the minimum dose of 17α-methyl testosterone in order to obtain an all male population of red tilapia (*Oreochromis niloticus*). Results of this study showed that each hormone treated group gave a mean male/female ratio that deviate significantly. The male population was significantly higher than females. The control group showed a 0.4:0.6 male:female ratio. In this study, the maximum male population (100%) of red tilapia was obtained in both 50 and 60 mg MT/kg of feed for 30 days. The minimum male proportion (60%) was recorded for a dose rate of 30 mg MT/kg of feed for 30 days. Obtained 97% *O. niloticus* males at dose rate of 10 mg MT/ kg of diet [13]. Other authors have used the higher dose rates to sex reverse *O. niloticus*. The obtained 99 to 100% males after treating fish for 25 days at 30 mg and 60 mg MT and 99% males with 30 mg of 17α-methyl testosterone (MT) fed for 21 days [14, 4]. + A 100% male sex population of Nile tilapia at 40 mg MT/kg of feed for each of 15, 20 and 25 days of treatment [16] and 100% masculinization of *O. niloticus* at a dose rate of 50 mg MT/kg of feed[16]. The dose rates both 50 and 60 mg MT/kg of feed resulted in 0.0% females. The dose of 60 mg 17α- methyl testosterone per kg of feed was found to be best for inducing sex reversal in *O. niloticus* resulting 94.28% male in the population [17]. Similarly, in the present study both 50 and 60 mg MT/kg resulted 100% male population. Thus a dose of 50 mg/kg could be considered for masculinization in red tilapia.

Growth Performance: In the present study, different doses of MT significantly influence the growth of red tilapia. As such the higher values for average body weight and gain in body weight of red tilapia were in treatments than the control clearly indicating that MT treatment enhances the growth rate of red tilapia very efficiently. The treatment group that received 60 mg MT/kg of feed (having the highest male percentage of 100%) showed the highest total net weight gain of 249.81gm. Observed faster growth of hormone treated *O. niloticus* [14]. The MT 10-60mg treatment had the best growth compared to the control [18]. The Observed faster growth rate in *O. mossambicus* when fed on MT treated diet and average total length and gain in total length also showed a marked difference among different treatments. The MT given treatments showed more gain in total length than controls [19, 20]. In the present study, the treatment T_4 (60 mg MT) showed the highest gain in total length (14.35 cm) as compared to controls (14.19 cm).

The specific growth rate of red tilapia ranged between 3.96% (control) to 4.48% (T_4 - 60 mg MT/kg of feed). These findings are in accordance with the findings who worked on the comparative study of growth and feed utilization efficiencies of sex reversed diploid and triploid Nile tilapia [21]. They recorded specific growth rate that ranged between 2.13-2.23% for Nile tilapia.

Survival of *O. niloticus* ranged from 61.33 to 77.33% during the grow out phase of the present study. Data showed no significant relationship between survival and MT treatment concentration. No significant effect of MT administration on survival of *O. niloticus* [22, 15, 3]. Compared the culture performance of different strains of *O. niloticus* and found survival rates in all treatments between 70.1 to 82.5% [23]. Further, they noticed a no significant difference between MT treatment and survival. The findings of present study are similar to these observations with regard to survival (73.3 - 76.0%) of *O. niloticus* and MT treatments [21].

Water Parameters: The water quality status monitored throughout the experimental period is summarized in Table 3. The parameters such as pH, dissolved oxygen (mg/l), total dissolved solids (mg/l), nitrate-nitrogen (mg/l) and EC (mMho) were measured and found congenial for the growth red tilapia. DO concentration should remain above 4 mg l^{-1} and the optimum temperature between 26- 28 ºC for ideal fish culture. The values of DO and temperature remained well within this limit which could be considered good for tilapia growth [22]

Table 1: Percent value of male and female populations in hormone treated red tilapia

Treatments	Sample size (Nos)	Male		Female		Sex ratio (M:F)
		Nos	%	Nos	%	
Control (0mg/kg)	30	12	40.00^d	18	60.00^a	0.4:0.6
T_1 (30mg/kg)	30	18	60.00^c	12	40.00^a	0.6:0.4
T_2 (40mg/kg)	30	23	76.67^b	17	23.33^c	0.77:0.23
T_3 (50mg/kg)	30	30	100.00^a	0	0.00^b	1:0
T_4 (60mg/kg)	30	30	100.00^a	0	0.00^b	1:0

Mean in the column with same superscripts are not significantly different

Table 2: Growth parameters of red tilapia fed with feed experimental diet

S. No.	Treatments	Control	Treatment T_1	Treatment T_2	Treatment T_3	Treatment T_4
1	Initial weight (g)	0.3	0.3	0.3	0.3	0.3
2	Net weight gain (g)	113.91±5.242^{e}	133.72±5.926^{d}	175.02±7.249^{c}	197.18±2.319^{b}	249.81±8.236^{a}
3	Per cent gain (%)	37868.56^{e}	44472.22^{c}	58241.44^{d}	65626.78^{b}	83171.44^{a}
4	Specific growth rate (%)	3.96±0.032^{e}	4.07±0.030^{d}	4.25±0.028^{c}	4.33±0.008^{b}	4.48±0.022^{a}
5	Total Length (cm)	14.19±0.16^{b}	14.23±0.01^{b}	14.25±0.01^{b}	14.34±0.07^{a}	14.35±0.02^{a}

Mean in the column with different superscripts are significantly different

Table 3: Range and average values (±Standard error) of selected water quality parameters during experimental period

Parameters	Water quality for treated tank	Water quality for untreated (control) tank
Temperature (ºC)	19.45-28.4 (25.07±0.87)	19.45-28.35 (25.14±0.90)
DO (mg/l)	5.25-8.06 (6.63±0.26)	5.48-7.93 (6.69±0.24)
TDS (mg/l)	982.0-1070.0 (1022.25±9.04)	914-1066.5 (1013.05±15.17)
EC (mMoh)	1.97-2.27 (2.06±0.03)	1.60-2.13 (1.97±0.06)
pH	7.14-8.37 (7.95±0.14)	7.1-8.42 (7.77±0.15)
Nitrate-nitrogen (mg/l)	0.015-0.027 (0.020.001)	0.016-0.045 (0.027±0.003)

Data are represented as means of six samples ± SE

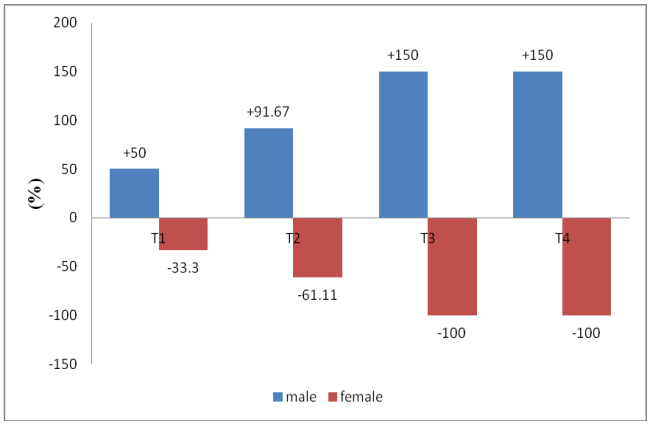

Fig 1: Per cent change in male and female populations over control in hormone fed fish

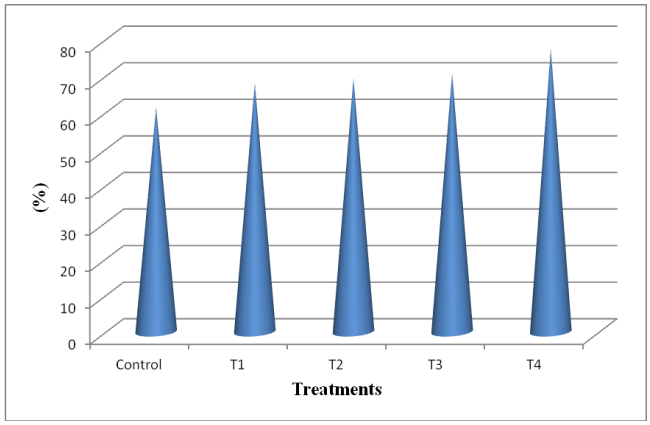

Fig 2: Survival in different treatments

Conclusion

The data obtaind on growth parameters (i.e. weight gain, length gain and SGR) of orally administered 17 α Methyl testosterone of red tilapia clearly indicated better performance of all male. Thus for the sustainable and higher growth the culture of all male red tilapia in recommend.

Acknowledgement

The author is thankful to Dr V.P.Saini Professor, Aquaculture Research Unit Directorate of Research, MPUAT, Udaipur, for their kind encouragement and cooperation for extending necessary facilities for conducting this research work smoothly.

Reference

1. Lester LJ, Lawson KS, Abella TA, Palada MS. Estimated heritability of sex ratio and sexual dimorphism in tilapia. Aquaculture Fisheries Management. 1989; 20:369-380.
2. Baras E, Melard C. Individual growth patterns of juveNile Nile tilapia, *Oreochromis niloticus* (L.): emergence and dynamics of sexual growth dimorphism. In: Fitzsimmons, K. (Ed.), Tilapia Aquaculture. NorthEast Regional Agriculture Engineering Service. 1997; 106:169-177.
3. Guerrero RD. III. Use of androgens for production of all male *Tilapia aurea* (Steindachner). Transactions of American Fisheries Society. 1975; 2:342-347.
4. Guerrero RD, III. Guerrero LA. Feasibility of commercial production of Nile tilapia fingerlings in Philippines. In: The Second Symposium on Tilapia in Aquaculture. ICLARM Conference Proceedings (ed. by R.S.V. Pullin, T. Bhukaswan, K. Tonguthi and J. L. Macleen), International Center for Living Aquatic Resource Management, Manila, Philippines. 1988; 15:183-186.
5. Abidi SZA, Bhatti MN, Majeed A. Effect of orally administrated 17- alpha-methyl testosterone on sex reversal and growth of *Oreochromis niloticus*. Proceeding of Pakistan Congress of Zoology. 1992; 12:317-323
6. Abucay JS, Mair GC. Hormonal sex reversal of tilpias: Implications of hormone treatment application in closed water systems. Aquaculture Research. 1997; 28(11):841-845.
7. Arthur JR, Lavilla-Pitogo CR, Subasinghe RP. Proceedings of the Meeting on the Use of Chemicals in Aquaculture in Asia. Southeast Asian Fisheries Development Center. Aquaculture Department, Tigbauan, Iloilo, Philippines, 1996, 244.
8. APHA. Standard methods for the examination of water and waste water. APHA, AWWA WPFC, 17th Ed. New York, 1989, 1452
9. Maar A, Mortimer MAE, van der Lingen I. Fish culture in Central East Africa. FAO Publications, Rome, Italy, 1966, 158.

10. Romerio MP, CS, Fenerich-Verani N, Santo De-Campos BE, Da-Silva AS. Masculinization of Nile tilapia, *Oreochromis niloticus*, using different diets and different doses of 17-alpha methyl testosterone. Revista Brasileira de Zootecnia. 2000; 29(3):654-659.
11. Smith ES, Phelps RP. Impact of feed storage conditions on growth and efficiency of sex reversal of Nile tilapia. North American Journal of Aquaculture. 2001; 63(2):242-245.
12. Okoko M. Effect of 17-alpha methyl testosterone concentrations on the sex ratio and gonadal development of Nile tilapia, *Oreochromis niloticus*. Masters Thesis, Auburn University, Alabama, US, 1996.
13. Jae-Yoon J, Smitherman RO, Behrennds LL. Effect of dietary 17 alpha methyl testosterone on sex reversal and growth of *Oreochromis niloticus*. In: The Second Symposium on Tilapia in Aquaculture. ICLARM Conference Proceedings (ed. by R.S.V. Pullin, T. Bhukaswan, K. Tonguthai and J.L. Macleen) 15, 623. Department of Fisheries, Bangkok, Thailand and International Center for Living and Aquatic Resources Management Manila, Philippines, 1988, 203-207.
14. Tayamen MM, Shelton WL. Inducement of sex reversal in *Sarotherodon niloticus* (Linnaeus). Aquaculture, 1978; 14(4):349-354.
15. Vera Cruz EM, Mair GC. Conditions for effective androgen sex reversal in *Oreochromis niloticus* (L.). Aquaculture. 1994; 122:237-248..
16. Bhandari RK, Nakamura M, Kobayashi T, Nagahama Y. Suppression of steroidogenic enzyme expression during androgen induced sex reversal in Nile tilapia (*Oreochromis niloticus*). General and Comparative Endocrinology. 2006; 145(1):20-24.
17. Ferdous Z, Ali MM. Optimization of hormonal dose during masculinization of tilapia (*Oreochromis niloticus*) fry. Journal of Bangladesh Agriculture University, 2011; 9(2):359-364.
18. Hanson TR, Smitherman RO, Shelton WL, Dunham RA. Growth comparisons of monosex tilapia produced by separation of sexes, hybridization and sex reversal.. [In L. Fishelson and Z. Yaron, eds.] International Symposium on Tilapia in Aquaculture, Proceedings. Tel Aviv University, Tel Aviv, Israel, 1983, 570-579.
19. Howerton RD, Okimoto DK, Grau EG. The effect of orally administrated 17 alpha methyl testosterone and triiodothyronine on growth and proximate body composition of sea water adapted tilapia (*Oreochromis mossambicus*). Aqaculture Fisheries Management. 1992; 23:123-128.
20. Varadaraj KS, Kumari S, Pandian TJ. Comparison of conditions for hormonal sex reversal of Mozambique tilapias. The Progressive Fish Culturists. 1994; 56:81-90.
21. Pechsiri J, Yakupitiyage A. A comparative study of growth and feed utilization efficiency of sex reversed diploid and triploid Nile tilapia, *Oreochromis niloticus*. Aquaculture Research. 2005; 36:45-51.
22. Soto P. Effect of mestanolone (17 α-methylandrostan - 17 ß-ol-3-one) on sex ratio and growth of Nile tilapia (*Oreochromis niloticus*). Masters thesis. Auburn University, Alabama, US: 1992, 58.
23. Dan NC, Little DC. The culture performance of monosex and mixedsex new-season and overwintered fry in three strains of Nile tilapia (*Oreochromis niloticus*) in northern Vietnam. Aquaculture. 2000; 184:221-231.
24. Phelps RP, Popma TJ. Sex reversal of tilapia. [In B.A. Costa-Pierce and J.E. Rakocy, eds. Tilapia Aquaculture in the Americas, The World Aquaculture Society, Baton Rouge, Louisiana, United States.] 2000; 2:34-59.