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# Dietary effects of 17α-methyltestosterone on masculinization in dwarf gourami trichogaster lalius

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**Abstract**
In the present investigation, 17α-methyltestosterone (MT) treatment at 50, 100, 150 and 200 ppm (mg/kg feed) to 3-day-old *T. lalius*, significantly produced highest masculinization than the control. At the highest dose (200 ppm) of MT produced maximum male percentage (85.32%). Indirect feminization also observed through crossing between sex reversal males with ordinary female. The GSI and histological examination showed the appearance of suppressed atretic oocytes. The hormonal profiling clearly revealed that, the MT treatment induced stress and produce the cortisol, which actively involved in the testosterone production and estradiol reduction.

**Keywords:** masculinization, *Trichogaster lalius*, 17α-methyltestosterone, progeny testing, gonadosomatic index, hormonal profiling

## 1. Introduction
Gonadal sex differentiation (estrogens and androgens) in fishes depends on endocrine cues even though, genetical sex determinations are considered as key tools [1-5]. Exogenous steroids when used at the appropriate time and dose i.e. early sex differentiation (labile period) of fishes helps to induces efficient sex reversal [6, 7, 4, 8]. The commercially available hormones include estradiol and 17α-methyltestosterone achieved greatest success of fish sex manipulation [9, 6, 10, 11]. The exogenous oral or immersions of steroids are mainly practiced as the mode of administration [12, 6, 13, 5]. The oral administration is probably the most effective method for sex manipulation in fishes as the labile period coincides with the external feeding fry and juvenile stages [14]. In recent years, especially in the ornamental fish industry, the hormonal manipulation of sex is receiving greater attention due to superior ornamental value of one sex for commercialization. Aquarium fish keeping is increasingly becoming popular in India. Apart from the domestic market, ornamental fishes also command good export potential. Therefore, there is tremendous scope to step up foreign exchange earnings of the country through this trade. Hence, the present study focused on masculinization in Dwarf gourami *T. lalius* because males have diagonal stripes of alternating blue and red colors and females have only silver color and hence, males gain high economic value. Therefore, production of all-male population would enhance the economic gains for ornamental fish enterprisers. The androgens especially MT hormone has long been utilized for induction of male population in many species of fish. Hence, hormonal induction of masculinization is important and interesting subject for this species. The objective of this study was to develop a protocol for masculinization in *T. lalius* through oral administration and to evaluate the effectiveness of the selected doses and treatment duration of MT on survival, sex reversal and gonad.

## 2. Materials and Methods
The study was performed, to investigate the effect of 17α-methyltestosterone on masculinization potential in *T. lalius.* The different doses of 17α-methyltestosterone (50, 100, 150 and 200 ppm) was prepared in Hikari fry feed, Japan by alcohol evaporation method [15] and selected doses were fed in triplicates to 3-day old fry *ad libitum* three times daily for 50 days. The control groups were fed without hormonal concentration. The 50 days MT treatment was conducted in glass aquaria and followed by rearing (with zooplankton) in FRP tanks.

In order to identify sex determination system in dwarf gourami, matured 3 males form all treatments including control were mated with ordinary females and the obtained fry were reared with zooplankton in FRP tanks for 160 days and followed by sexing to examine the sex ratio.

The matured treated and control fishes were sacrificed for hormonal analysis different tissues like gonads and liver were dissected and the different hormone levels like testosterone and estradiol were tested as per the protocol of respected EIA kits (Cayman Chemicals, USA) whereas cortisol extraction was performed by [16, 17, 18]. Body weight and gonad weight were recorded for calculation of the gonadosomatic index (GSI; gonad weight expressed as percentage of the body weight). To study the histology of gonads, samples from MT treatments were fixed using 10% neutral buffered formalin. The samples were then processed using a graded series of alcohol, embedded in paraffin wax and the sections of 5µm thickness were cut using a microtome and spread on slides, the sections thus obtained were stained with Delafield's haemotoxylin – Eosin stain.

**Data Analysis**

Differences in survival, male percentage, gonadosomatic index and hormonal profiling among treatment were subjected to one way ANOVA with Duncan's multiple range test (SPSS 18).

**3. Results and Discussion**

The current study successfully achieved efficient masculinization in *T. lalius* through MT oral administration for 50 days (Table 1). The highest percentage of males was observed 85.32% (200 ppm) with 91.61% survival whereas in other groups, the males percentage were 48.73, 49.09, 56.07 and 75.16% at 0, 50, 100 and 150 ppm respectively, with the survival of 87.29, 91.72, 92.41 and 91.92% respectively. During treatment period, the survival was 55.83, 50, 49.16, 45.83 and 49.58 at 0, 50, 100, 150 and 200 ppm respectively which indicated that as the MT dose increases, the males percentage increases and survival rate decreases significantly (<0.05) than that of the control group. In general, a higher percentage of males were observed in all the treated groups across all the treatments, compared to the control (Table 1).

The results of the present study investigated that it is possible to induce the sex ratio of dwarf gourami through oral administration of a synthetic androgen, MT, when administered at 50–200 mg/kg diet to first feeding fry for 50 days, produced 49.09 to 85.32% male populations whereas control group reported lower proportion of male population (48.73%). Similarly, previous study also reported up to 84.29% males in dwarf gourami after immersion with an androgen, MT [19] which suggested that MT is a more efficient chemical for sex manipulation in *T. lalius*. Likewise, [20] successfully induced 59-100% males in silver perch, *Bidyanus bidyanus* by the dietary administration of an androgen, MT, at 9-18 mg/kg. In another study, MT at 60 mg/kg MT produced of up to 85.7% males in Blue Hap [21], and up to 81.90% masculinization achieved in *Poecilia reticulata* after dietary treatment for 30 days [22] but in this study more than 80% males were observed only at 200 ppm. This may be because differential distribution of MT in diet, differential intake or degradation of MT in the digestive system. Our findings are useful that male would not only help to overcome undesirable female production but also enhance profit due to higher market price. India's ornamental market may enhance with major contributions coming from monosex technology. Ornamental fish enterprisers will be expected to get higher yield and derive more profit by stocking all male dwarf gourami. This study will help entrepreneurs, particularly startups, aims to incorporate this protocol for the production of monosex male dwarf gourami using MT. The results of progeny testing of males from treated and normal males of oral are shown in Table 2. The males from MT fed groups when mated with normal females, produced 45.43 (control), 65.26, 75.42, 77.85 and 86.02% females. The percentage of male and female significantly ($p<0.05$) changed in each progeny group when compared with control group. Similarly, [19] showed that 100% female progeny could be produced by crossing sex-inverted male *T. lalius* with normal female. Likewise, in *B. splendens* also obtained an all-female progeny when sex-reversed males were mated with normal females [23]. However, in *T. lalius* confirmed the involvement of only the monofactorial system of sex determination, i.e. the XX-XO system [24]. The results of our study matched with their observations, confirming the involvement of XX chromosome in the female of *T. lalius*. However, further study may be needed to confirm the efficacy of 50-200 ppm MT. While [25] obtained 95.4% male progeny of Nile tilapia by oral administration of MT at 70 ppm for 25 days. Upto 98% males reported in *Xiphophorus hellerii* after feeding with MT at 75 mg/kg [26]. In recent years, sex reversal studies mostly focused on the use of aromatase inhibitors, our study has proved that oral administration of MT is more effective to produce a male-dominant dwarf gourami population. The MT treatment has no negative effects on general fish health. Similarly, increase in testosterone level was also reported in male *T. lalius* treated with MT [19]. Dietary treatment with MT was reported to cause significant increase in percentage of males in bay snook, *Petenia splendida* [27]. Dietary inclusion of MT at a concentration of 60 mg/kg diet have resulted in 95 and 97% male population for 75 and 365 days in *O. niloticus* [28] but in present study lower percentage of males were observed at 50, 100 and 150 ppm (49.09, 56.07 and 75.16 respectively) which suggested to increase the duration of treatment more than 50 days for effective masculinization at lower doses in *T. lalius*. In another experiment, 68% masculinization was achieved in koi carp by dietary administration 90 days containing 50 ppm MT [29]. Results emanating from this study indicate a dose dependent masculinization effect of MT on dwarf gourami, which corroborates with other fish studies, where percentage of males increased with increase in the MT concentration. [29] obtained 80% sex reversed males in Platy fed diets containing 500 mg/l for 90 days. The results of the effect of MT treatment on gonodosomatic index of male and female are shown in Figure 1. The gonadosomatic index of male fish did not vary significantly between different groups. In the females, suppression of ovarian development was observed in all the treatments, compared to control. Suppression of GSI was observed in all treated groups as the doses of MT increased (Figure 1). Similar results were reported by [19] in *T. lalius*. On the other hand, no significant differences ($P>0.05$) was observed in gonadosomatic index (GSI) among MT treatments in silver perch, *Bidyanus bidyanus* [20]. Histological examination showed atretic oocytes in the MT treated groups which indicated suppression in ovarian development whereas in testicular development no adverse effect has been found (Figure 2). Several author reported the same trend of histology in sex reversal fishes, [23] observed that the letrozole suppressed the ovarian development in *B. splendens*. [20] also reported normal testicular development in MT-fed silver perch fish. Slightly

suppressed testicular development and completely inhibited ovarian development was observed in norethindrone (androgen) treated groups of common carp [30]. The cortisol levels of the oral treatment of MT are presented in Figure 3. More or less similar cortisol level was observed in male treated and control groups while the female cortisol level increased as doses increased. Cortisol is an indicator of stress has a direct and indirect relation with maturing hormone testosterone and estradiol; as the increased in cortisol level leads to increase in testosterone also and decrease estradiol. Cortisol is the main component associated with stress in fishes [31]. Recent studies indicated that cortisol initiating a shift in steroidogenesis from estrogens to androgens. [19] reported MT immersion treatment imposed stress in *T. lalius*. In the present study also MT induced the stress in female for sex reversal.

The results of testosterone levels are presented in Figure 4. In our study, MT was found, to stimulate testosterone production. Male testosterone level indicated that as the doses of MT increased testosterone level also increased; similar observation were found in case of female testosterone level and helps to decreased level of estradiol as the MT doses increased (Figure 5).

In males, fadrozole treatment led to a significant increase in plasma concentrations of both T and KT. It probably inhibits CYP19 that would increase androgen concentrations in males by arresting conversion of T to E2 [32]. The lowest $E_2$ values were observed in Eurasian perch (*Perca fluviatilis*) that received fadrozole 100 mg/kg diet treatment [33].

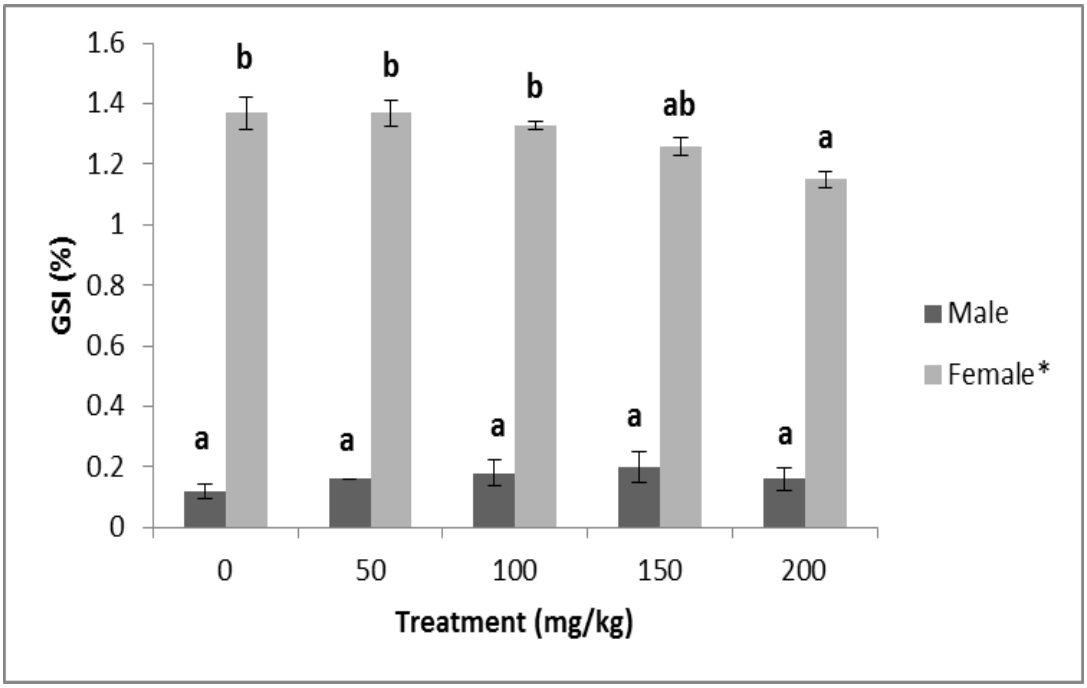
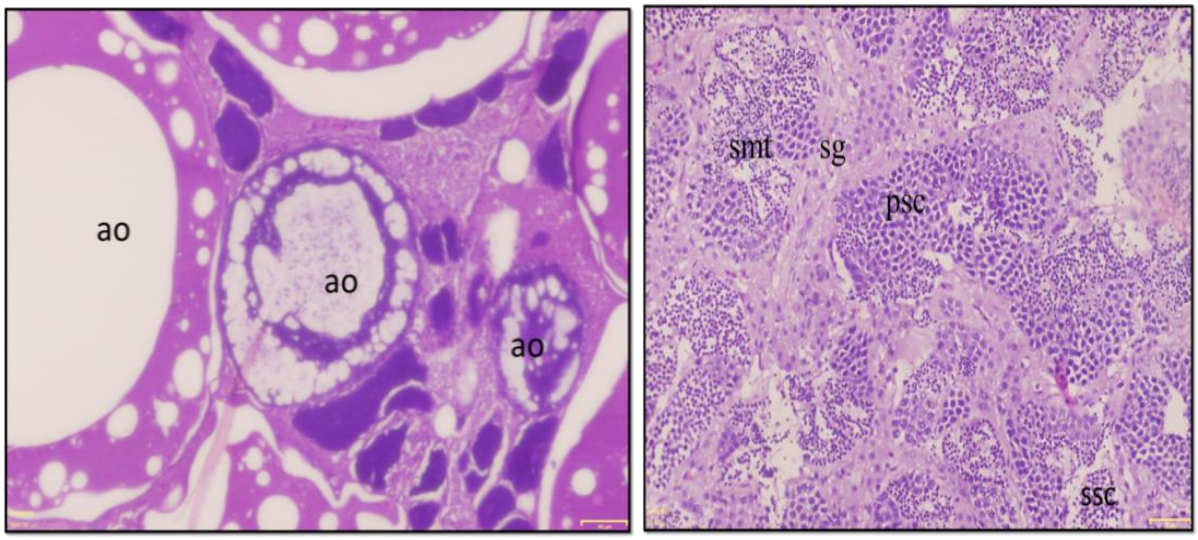



**Fig 1:** Gonadosomatic index of MT treated fish (Oral treatment) Data expressed as Mean ± SE n=3, *Mean values in the same column with different superscript differ significantly ($p$<0.05).




**A.** **B.**

**Fig 2:** Photomicrograph: MT treated fish showing A) Degenrated atretic oocytes (ao; Scale bar: 64 µm; X10). B) Mature testis (spermatogonia: sg, primary spermatocytes: psc, secondary spermatocytes: ssc, spermatids: smt and spermatozoa: sz. (Scale bar: 32 µm; X20).

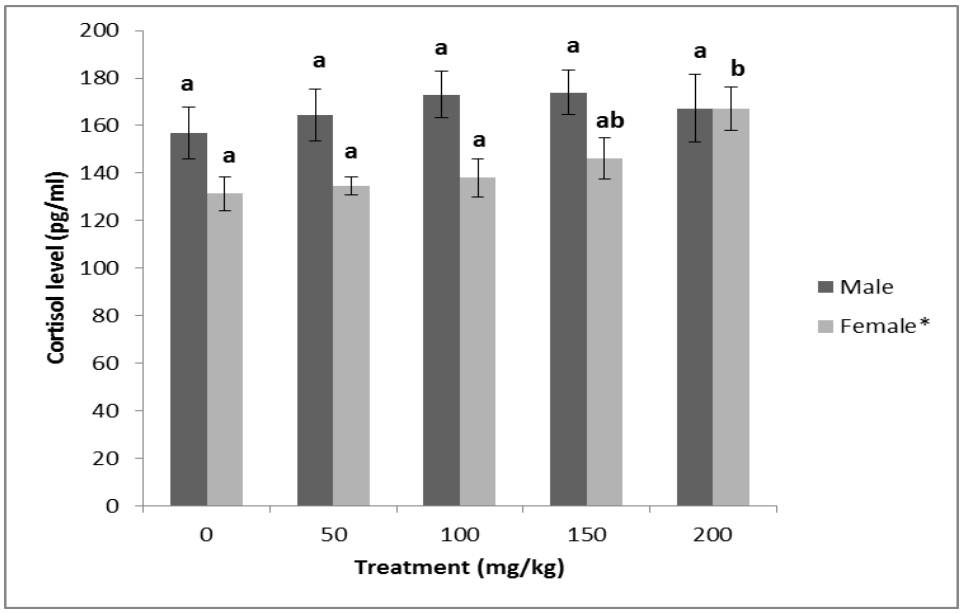



**Fig 3:** Cortisol level in MT treated fish (Oral treatment) Data expressed as Mean ± SE n=3, *Mean values in the same column with different superscript differ significantly ($p$<0.05)

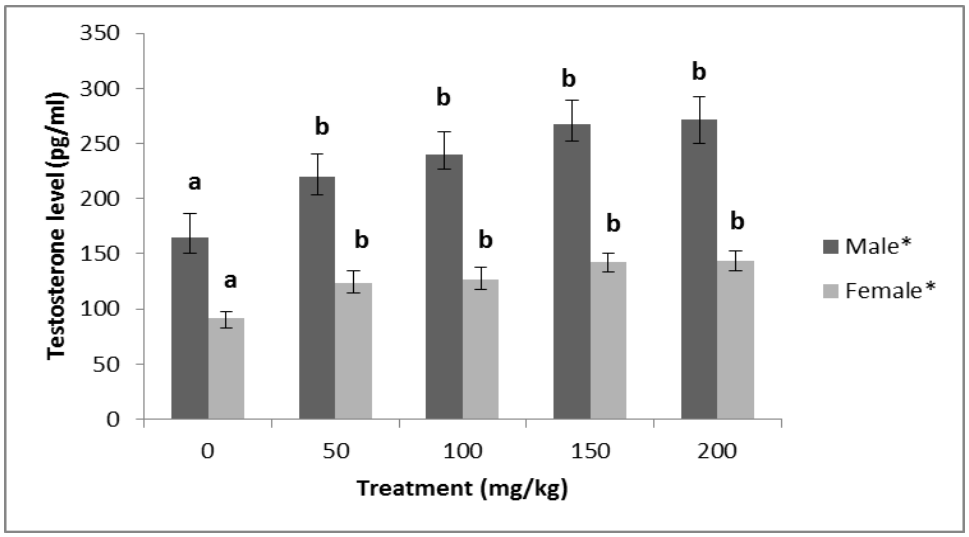



**Fig 4:** Testosterone level in MT treated fish (Oral treatment) Data expressed as Mean ± SE n=3, *Mean values in the same column with different superscript differ significantly ($p$<0.05).

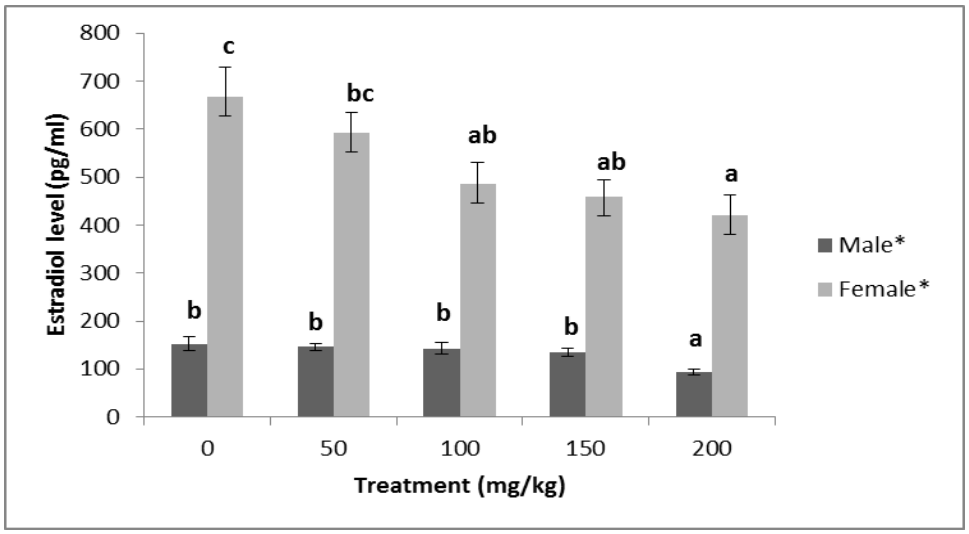



**Fig 5:** Estradiol level in MT treated fish (Oral treatment) Data expressed as Mean ± SE n=3, *Mean values in the same column with different superscript differ significantly ($p$<0.05).

**Table 1:** Number of fry stocked, recovered, sex composition and survival of *T. lalius* given oral treatment of 17α-methyltestosterone

| Treatment | Treatment Period | | | Post- treatment period | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | No. of fry (Initial) | No. of fry* (Final) | Survival (%)* | No. of fish recovered | Males* | Females* | Male (%)* | Female (%)* | Survival (%) |
| Control | 80 | 44.66±0.88$^{b}$ | 55.83±1.20$^{b}$ | 39.00±1.52$^{b}$ | 19.00±1.00$^{a}$ | 20.00±1.15$^{d}$ | 48.73±2.00$^{a}$ | 51.26±2.00$^{d}$ | 87.29±2.67$^{a}$ |
| 50 ppm | 80 | 40.00±0.57$^{a}$ | 50.00±0.72$^{a}$ | 36.66±0.33$^{ab}$ | 18.00±0.57$^{a}$ | 18.66±0.66$^{d}$ | 49.09±1.62$^{a}$ | 50.90±1.62$^{d}$ | 91.72±2.07$^{a}$ |
| 100 ppm | 80 | 39.33±1.45$^{a}$ | 49.16±1.81$^{a}$ | 36.33±1.20$^{ab}$ | 20.33±0.33$^{a}$ | 16.00±1.15$^{c}$ | 56.07±1.82$^{b}$ | 43.92±1.82$^{c}$ | 92.41±1.29$^{a}$ |
| 150 ppm | 80 | 36.66±1.76$^{a}$ | 45.83±2.20$^{a}$ | 33.66±1.20$^{a}$ | 25.33±1.33$^{b}$ | 8.33±0.33$^{b}$ | 75.16±1.14$^{c}$ | 24.83±1.46$^{b}$ | 91.92±1.19$^{a}$ |
| 200 ppm | 80 | 39.66±0.88$^{a}$ | 49.58±1.10$^{a}$ | 36.33±0.66$^{ab}$ | 31.00±0.57$^{c}$ | 5.33±0.33$^{a}$ | 85.32±0.80$^{d}$ | 14.67±0.80$^{a}$ | 91.61±0.69$^{a}$ |
| P value | | <0.05 | <0.05 | 0.067 | <0.05 | <0.05 | <0.05 | <0.05 | 0.288 |

*Data expressed as Mean ± SE, Mean values in the same column with different superscript differ significantly (p<0.05).

**Table 2:** Results of progeny testing of 17α-methyltestosterone treated and control groups (oral treatment)

| Treatment (ppm) | No. of fry (Initial) | No. of fry (Final) | Survival (%) | Males | Females | Male (%) | Female (%) |
|---|---|---|---|---|---|---|---|
| Control | 87.33±1.45$^{b}$ | 55.00±2.30$^{c}$ | 62.96±2.18$^{b}$ | 30.00±1.15$^{d}$ | 25.00±1.15$^{a}$ | 54.56±0.19$^{d}$ | 45.43±0.19$^{a}$ |
| 50 | 63.33±2.40$^{a}$ | 39.33±0.66$^{b}$ | 62.26±2.31$^{b}$ | 13.66±0.66$^{c}$ | 25.66±0.66$^{a}$ | 34.73±1.46$^{c}$ | 65.26±1.46$^{b}$ |
| 100 | 67.33±1.45$^{a}$ | 40.66±0.88$^{b}$ | 60.39±0.39$^{b}$ | 10.00±0.57$^{b}$ | 30.66±0.66$^{b}$ | 24.57±1.14$^{b}$ | 75.42±1.14$^{c}$ |
| 150 | 65.33±1.76$^{a}$ | 37.66±0.33$^{b}$ | 57.75±1.98$^{b}$ | 8.33±0.33$^{b}$ | 29.33±0.66$^{b}$ | 22.14±1.09$^{b}$ | 77.85±1.09$^{c}$ |
| 200 | 66.00±1.15$^{a}$ | 33.33±0.88$^{a}$ | 50.50±1.01$^{a}$ | 4.66±0.33$^{a}$ | 28.66±0.66$^{b}$ | 13.97±0.78$^{a}$ | 86.02±0.78$^{d}$ |
| P value | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 |

Data expressed as Mean ± SE n=3, Mean values in the same column with different superscript differ significantly (p<0.05)

## 4. Conclusion

The current study concluded that the optimum dose of MT required to produce all-male population of dwarf gourami is 200 mg/kg diet to be fed only for 50 days. This study also confirms the indirect feminization through mating sex reversal males with normal female. It reveal that MT supplementation induce cortisol and testosterone synthesis whereas estradiol and ovarian suppression. As the highest treatment concentration of 200 mg/kg feed produced the maximum percentage of males among the different treatment categories in this study, further analysis with increased concentration might be required to achieve 100% sex reversal with MT. Oral treatment with MT was found to significantly increase male percentage while to decrease survival.

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