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# Masculinization of silver perch (*Bidyanus bidyanus* Mitchell 1838) by dietary supplementation of 17α-methyltestosterone

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**KEYWORDS**

Masculinization;
Methyl-testosterone;
Silver perch;
*Bidyanus bidyanus*

**Abstract** The aim of this research was to assess the possible use of dietary supplementation of 17α-methyltestosterone (MT), to produce all-male population of silver perch, *Bidyanus bidyanus* Mitchell 1838, as a step forward in producing neomales, which later can be used to produce an all-female population. Larvae were fed 17α-MT at various concentrations, viz. 0 (control), 9 and 18 mg/kg diet for the period of 30 days from 31 to 60 days post hatching (dph). Phenotypic sex ratios at 225 dph identified through histological examination revealed that MT significantly ($P < 0.05$) increases the male percentage from 59% to 100%. Testes of MT-fed fish were well developed, had a normal appearance at the same developmental stages to that of the control group. No significant differences ($P > 0.05$) in gonad weight (GW), gonad length (GL) and gonadosomatic index (GSI) among treatments which may indicate that the resulting neomales were viable. The MT supplementation did not influence the mortality rate, but significantly ($P < 0.05$) increased the final weight and specific growth rate (SGR). The study suggests that the dietary supplementation of MT at 9–18 mg/kg of the diet from 31 to 60 dph larvae is effectively in inducing masculinization in silver perch.


## Introduction

The protandrous hermaphrodite silver perch (*Bidyanus bidyanus*, Mitchell 1838) is recognized as an important cultivated species in Australia. Faster growth is an important factor for profitable aquaculture. Several ways have been tried to increase silver perch aquaculture production but stunted growth, and precocious maturation has halted the anticipated productivity (Gordon, 1995). Silver perch exhibits sexual dimorphism, where males are smaller than females

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(Mallen-Cooper, 2003). Most silver perch males induce maturation at two years of age and thus divert their energy resources into gonadal development. On the other hand, females still use the resources for somatic growth and mature at three years, when they are just approaching marketable size, at 1 kg (Rowland, 2004). Hence, females have one additional year than males in which they can divert their energy resources into somatic growth. Consequently, since females do not mature before harvest size and grow bigger than males, to obtain an all-female population is an economical choice for silver perch aquaculture.

Hormonal sex control has been successfully applied for the direct feminization or masculinization in a substantial amount of fish species (Pandian, 2013; Pandian and Sheela, 1995). However, no published paper related to sex reversal on silver perch has been reported. Our previous study (unpublished data) suggested that direct feminization was successfully induced in all-female population by the used of estradiol 17-β hormone. It is also well known that all-female populations can be achieved by indirect feminization through mating functionally sex-reversed females (neomales) with regular females (Devlin and Nagahama, 2002; Donaldson, 1996; Pongthana et al., 1999). The induced sex reversal of XX male sex genotypes in the latter instance is a means of producing monosex all-female population of fish under XY sex-determining system, as expected for silver perch. Even though more time and labour consuming than the direct feminization, the indirect method has an advantage where harvested fish have never been treated with steroids hormone. Therefore, its application would avoid a clash with the restriction of using hormones in fishes produced for human eating purpose. Furthermore, the indirect application of hormone is also recognized as a beneficial tool in identifying the homogametic sex (Blázquez et al., 1995; Liu et al., 2013; Mei and Gui, 2015). Another method that may be simpler is cytogenetic determination of sex chromosomes as sex-linked markers which have been identified in a few species (Dan et al., 2013; Pan et al., 2015; Wang et al., 2009; Zhang et al., 2016).

The masculinization technique using hormone intended to produce neomale fish, has been applied in numerous fish species, for example yellow fin perch, *Perca flavescens* (Malison et al., 1986); rainbow trout, *Oncorhynchus mykiss* (Cousin-Gerber et al., 1989); Nile tilapia, *Oreochromis niloticus* (Mair et al., 1991), common carp, *Cyprinus carpio* (Gomelsky et al., 1994); and redfin perch, *Perca fluviatilis* (Rougeot et al., 2002). However, an inconsistent increase in percentage of male resulting from hormonally treated fish has also been mentioned by Blázquez et al. (2001) in several fish species such as channel catfish, *Ictalurus punctatus*; rainbow trout; coho salmon, *Oncorhynchus kisutch*; and Chinook salmon. As the influence of sex androgen on gonadal differentiation is species specific (Piferrer, 2001), the timing and optimum dosage of hormonal treatment for targeted cultivated species, including silver perch, need to be investigated.

An important step in establishing an effective regime of hormonal usage to masculinize fish is the identification of the 'labile period' i.e. the period where gonad is highly sensitive to the exogenous factors, including treatment of steroids (Piferrer, 2001). In silver perch, the gonads are anatomically formed in 30-day fry but cytological differentiation occurs only after the 60-day old larva stage (Moiseeva, 2001). Therefore, in this study, we introduced androgen treatment for masculinization at 31–60 dph.

The present research aims to assess the potential of two concentrations of dietary supplementation of 17α-MT to produce an all-male population of silver perch as a step of producing neomales which later can be used to produce an all-female population.

## Material and methods

This experiment has been approved by the Animal Ethics Committee of Curtin University (approval number AEC_2011_70). Besides, the Australian Code of Practice for the care and use of animals for scientific study was also followed.

### *Preparation of the MT-containing diets and handling*

The MT powder (Sigma, M-7252) was reconstituted in 95% ethanol (1 mg/L) to prepare a stock solution before being incorporated into manufactured feed using alcohol saturation methods (Hendry et al., 2003) and evaporation methods (Lin et al., 2012; Rougeot et al., 2002). Commercial feed (spectrum micron diets, NRD® 2/4 200–400 μm; protein: 55%, crude fat: 9%, fibre: 1.9%; INVE-Thailand) was saturated with 50 mL MT-ethanol solution in petri dishes (each of 20 mg feed). The concentrations of 9 and 18 mg MT/kg diet need 0.18 and 0.36 mL of 1 g/L MT, respectively. The control feed was saturated with ethanol only. The diets were dried overnight under a fume hood before being kept at 4 °C until further use.

The MT-supplemented and control diets were fed in triplicate to silver perch juveniles in the nine prepared glass aquaria. The diets were given manually to 31–60 dph silver perch, until satiation, three times per day during daylight hours. The post 60 dph fish were then fed an untreated artificial diet (NRD G8, 0.8 mm) until termination of the experiment at 225 dph.

### *Broodstock handling*

The domesticated silver perch broodstock, which had been maintained in a 10-ton fibreglass tank for approximately six years at Curtin Aquaculture Research Laboratory (CARL), Western Australia, was used to produce fry. Only mature broodstock were selected where in male releasing milt on pressure while in female showing oocyte at about 1 mm in diameter (Rowland, 2004). Male and female broodstock with total length (TL) and body weight (BW) of 510 mm and 2.4 kg and 480 mm and 3.8 kg respectively were injected with human chorionic gonadotropin (hCG) hormone at a dose of 200 IU/kg fish (Levavi-Sivan et al., 2004; Rowland, 2009) to initiate spawning. After hormonal injection, the broodstock were maintained in a two-tonne cylindrical fibreglass tank at room temperature (20–26 °C) until they spawned.

### *Experimental fish preparation and maintenance*

About 10,000 hardened eggs were transferred into incubator tanks an hour after the spawning. The incubator tanks were designed as a flow-through system equipped with a sump tank to hold newly hatched larvae, which were then transferred to a

300-L circular tank (as a holding tank) at a stocking density of 50 larvae/L. Water salinity in the holding tank was increased to 6 ppt to optimize larval survival. The holding tank was gently aerated and equipped with a heater (Sonpar, Model-Ha200) to maintain the temperature at 20 ± 1.0 °C. Initially, fish larvae were fed live rotifers and *Artemia* nauplii that were then replaced by a dried formulated diet (NRD 2/4) at 15 dph and occasionally supplemented with *Artemia* nauplii until 25 dph.

Randomly chosen larvae at 26 dph, at a density of 50 larvae per aquarium, were placed in nine 10-L glass aquaria filled with filtered fresh water (Aqua-pure Model-AP12S, 5 microns). The aquaria were arranged in an incubator bath equipped with a heater (stirred thermostatic circulator, Model-GD120) to keep the water temperature stable at 23 °C. In order to minimize light penetration into the water column in the aquaria, each aquarium was wrapped with a black plastic sheet. An air stone was installed in the middle of each tank for DO diffusion and food dispersion. During the acclimation period, the dead larvae were replaced with new larvae obtained from the holding tank. From 26 to 30 dph, only NRD 2/4 feed was provided. Two weeks after the completion of hormonal treatment, all fish from each experimental unit were transferred to a separate 200-L plastic container to keep treatments and replicates separated. The containers were designed in a flow-through system where temperature and oxygen concentration were maintained at 23 ± 2 °C and 7 ± 1 mg/L, respectively.

### *Data collection and sex determination*

Fish body weight (BW, precision 0.01 g) was regularly monitored at 1, 75, 105, 135, 165, 195, and 225 dph, but total length (TL, precision 1 mm) was only measured at the commencement and at the termination of the experiment. Fish were anaesthetized using AQUI-S (0.025 mg/L) prior to weighing and measuring TL individually during sampling. At the end of the experiment, all fish were killed with an overdose of AQUI-S (100 mg/L). Gonads were removed and visually identified as ovaries or testes. Gonad weight (GW, precision 0.0001 g) and length (GL, precision 0.01 mm) were also measured. Survival was calculated by comparing the number of live fish at 225 dph with the initial number of fish, and the survival percentage was calculated. At the same time, fish sex was confirmed by the standard gonadal squash technique (Anonimous, 2009; Conover and Fleisher; 1986; Guerrero and Shelton, 1974).

At the end of the experiment, the gonads of all fish were fixed in formalin buffer solution (FBS), embedded in paraffin wax, sectioned at a thickness of 7 µm, and stained with haematoxylin and eosin (Luna, 1968). Slides were photographed under the microscope using an Olympus SC30 camera and GetIt software Olympus. The early stages of gonadal development were compared to published as described by Coward and Bromage (1998), Lin et al. (2012), Maack and Segner (2003), Almeida et al. (2008), and Lubzens et al. (2010).

The gonadosomatic index (GSI) was determined according to the formula suggested by Razak et al. (1999): GSI = (GW/BW) × 100, where GW and BW are in grams. Specific growth rate was calculated using the formula: SGR = ($e^g$ − 1) × 100% (Árnason et al., 2009; Hopkins, 1992), where $g$ = [ln ($w_2$) − ln($w_1$)]/($t_2$ − $t_1$), where $w_2$ and $w_1$ are the mean weights on day $t_2$ and $t_1$, respectively. Condition factor ($K$) was calculated as: $K = \mathrm{BW} \times \mathrm{TL}^{-3} \times 100$ (Jamet and Desmolles, 1994).

### *Statistical analyses*

Mean and standard errors (SE) were considered for BW and TL at each sampling times, and also for GW, GL, GSI and SGR at 225 dph for each dietary treatment. Data expressed in percentages were arcsine transformed to ensure normality prior to further analyses. The significant differences between treatments in each parameter determined by one-way ANOVA followed by Tukey's post hoc (multiple comparisons) tests. SPSS 22.0 package was used for all statistical analyses. Differences were accepted as significant when $P < 0.05$. Only male fish were included in the control group.

## Results

### *Sex differentiation and masculinization*

There were no visual deviations, such as lordosis or superficial blackening of the skin, in fish from any treatment groups. Sex identification through the examination of gonad morphology enabled the separation of male from the female fish. There was no discrepancy, between morphological identification of gonads, wet-squash and the histological examinations of gonads in the identification of sexes. Morphological differences between ovaries and testes were clear. A pair of ovaries in females and a pair of testes in males had similar transparency or whiteness in colour but had different shapes, in that the ovaries were rounded and the testes were trianguar under cross-section examination (Fig. 1A and D). Female gonads from the control groups were arranged in lamellae containing oocytes with lightly stained nuclei, surrounded by darker cytoplasm (Fig. 1B). Testicular tissue from all treatments with early beds of developing spermatocytes was present in the lobule tissue (Fig. 1E).

In addition, histological analysis of females from the control groups had immature ovaries containing oocytes and oogonia (Fig. 1C). The testes from MT-treated and control groups (Fig. 1F) however, consisted of testicular lobules with the different stage of male gametes such as spermatogonia, spermatocytes, and spermatid. All female gonads showed the ovarian lamellae projecting into the lumen, whereas male gonads showed the formation of the cyst in the testicular tubules.

The testes of all male fish fed MT were well developed, similar to those of the control groups. No indication of abnormalities such as gonadal retardation, intensification of connective tissue, lack of germinal tissue, and lack of testicular tubules, as commonly found in sterile gonads, were observed. There was no sign of ovarian degradation, which showed that no males had ever gone through female stages in their life histories. Contrary to this, the females from the control population showed visible clusters of degraded male cells, demonstrating that they first went through male stages before changing into females (protandrous hermaphrodite), as described by Moiseeva (2001). Fish fed the MT-diet developed similar gonads, in terms of weight and length, to the fish fed control diet.

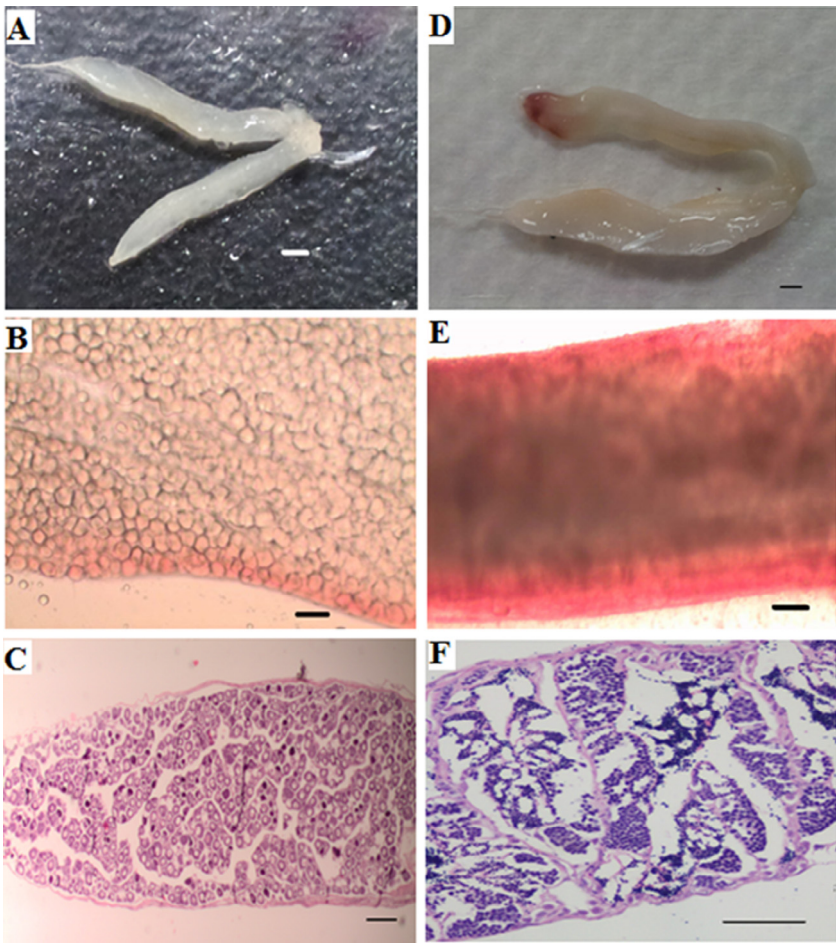


**Figure 1** Gonad visualisation in silver perch, *Bidyanus bidyanus* juvenile at 225 dph. (a) Photomacrograph of an ovary showing pale white colour and rounded cross-section; scale bar = 10 mm. (b) Wet-squash preparations of an ovary with previtellogenesis oocytes; scale bar = 100 µm. (c) Histological section of an ovary with oocytes located in the ovarian lamellae; scale bar = 100 µm. (d) Photomacrograph of testis showing colour similar to that of ovary but triangular in cross-section; scale bar = 10 mm (e) Wet-squash preparations of testis showing typical testicular tubules; scale bar = 100 µm (f) Histological section of testis showing spermatogonia in the testicular tubules; scale bar = 50 µm.

The lack of females, as opposed to 40% of females in the control group were induced by dietary MT supplementation. MT supplementation either at 9 mg/kg or 18 mg MT/kg diet resulted in the induction of a significantly ($P < 0.05$) higher percentage of males compared to control, which shows the complete masculinization of the silver perch (Fig. 2).

*Growth*

Fish fed MT had significantly ($P < 0.05$), higher weight gains but showed no significant ($P > 0.05$) differences in GSI (Table 1). Fish weight increased steadily after slow growth during the first two and a half months (Fig. 3). There were no significant ($P > 0.05$) differences in mean weight at 75 dph or at two weeks after the withdrawal of dietary supplementation. However, at the end of the experiment (225 dph), the total weight of fish fed MT was significantly ($P < 0.05$) higher than that of the control groups (Fig. 3). Food efficiency (weight gain/food intake) was significantly higher in MT-fed fish than in control groups (Table 1).

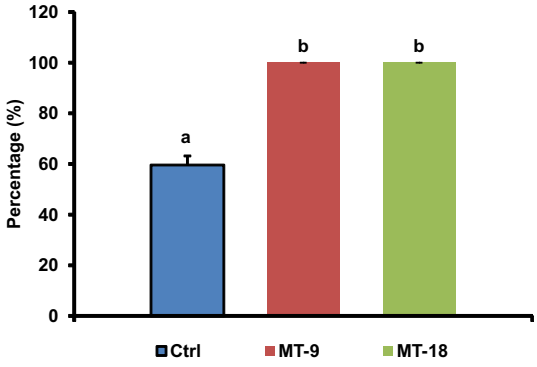


**Figure 2** Proportion of males of silver perch at the end of the experiment. Ctrl, provided feed saturated with ethanol only; MT-9, MT fed at 9 mg/kg diet; MT-18, MT fed at mg/kg diet. Different letter for each treatment indicate that the means are significantly different at the 0.05 level.

**Table 1** Effects of hormonal (MT) treatments on survival rate, specific growth rate, and food conversion efficiency in silver perch, *Bidyanus bidyanus*.

| Parameters | Treatment | | |
|---|---|---|---|
| | Control | 9 mg/kg diet | 18 mg/kg diet |
| Initial weight (g) | $0.18 \pm 0.03^{a}$ | $0.17 \pm 0.03^{a}$ | $0.20 \pm 0.03^{a}$ |
| Survival rate (%) | $39.33 \pm 3.33^{a}$ | $51.33 \pm 1.33^{ab}$ | $44.42 \pm 2.40^{b}$ |
| Final Weight (g) | $11.52 \pm 0.98^{a}$ | $19.62 \pm 1.49^{b}$ | $17.60 \pm 0.81^{b}$ |
| Gonad weight (g) | $0.06 \pm 0.02^{a}$ | $0.07 \pm 0.02^{a}$ | $0.06 \pm 0.03^{a}$ |
| Gonad length (mm) | $18.79 \pm 3.22^{b}$ | $21.07 \pm 1.21^{a}$ | $22.60 \pm 1.90^{a}$ |
| Male percentage (%) | $59.6 \pm 3.6^{a}$ | $100.0 \pm 0.00^{b}$ | $100.0 \pm 0.00^{b}$ |
| GSI (%) | $0.37 \pm 0.10^{a}$ | $0.33 \pm 0.12^{a}$ | $0.33 \pm 0.15^{a}$ |
| SGR (%) | $3.3 \pm 0.04^{a}$ | $3.56 \pm 0.03^{a}$ | $3.51 \pm 0.02^{a}$ |
| FE | $29.78 \pm 1.00^{a}$ | $34.76 \pm 0.47^{b}$ | $33.18 \pm 0.89^{b}$ |

Note: Means with different superscripts in the same row are significantly different at *P*-0.05 (Tukey test). GSI, gonadosomatic index.

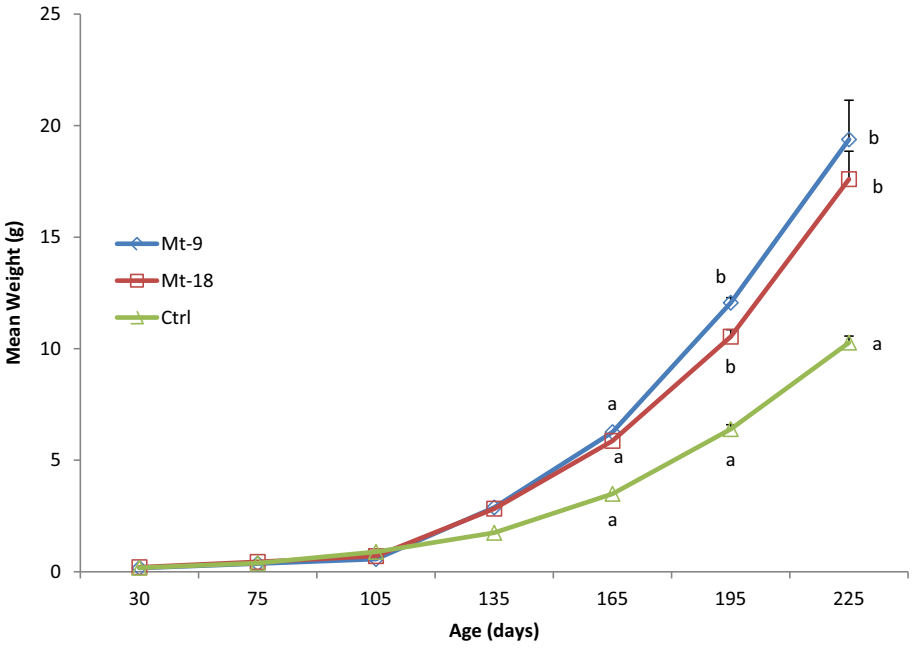


**Figure 3** The increase in body weight of silver perch, *Bidyanus bidyanus* juvenile fed MT. Vertical bars indicate SE. Mt-9, MT fed at 9 mg/kg diet; MT-18, MT fed at 18 mg/kg diet; Ctrl, provided feed saturated with ethanol only. Sample sizes of 7–12 were used for each replicate, and different letters for each age indicate that the means are significantly different at the 0.05 level.

*Mortality*

At the end of experiment, survival in all treatment ranged from 39% to 51% (Table 1), with most of the mortalities arising in the beginning period of the experiment: that is at the commencement of the feeding on formulated diet. The survival of silver perch was independent of the dietary MT dosage.

## Discussion

The doses (0, 9, and 18 mg/kg diet) of MT hormone used in this experiment were based on the supplementation level applied to the other species such as rainbow trout, *Salmo gairdneri* (Solar et al., 1984); European sea bass, *Dicentrarchus labrax* (Blázquez et al., 2001); and golden barb, *Puntius gelius* (Montajami, 2012). The production of all-male or all-female populations through sex reversal in aquaculture operation is one way of directing feed-derived energy into growth. There are three main variables involved in the success of sex reversal: (i) the timing of hormonal treatment in relation to gonadal improvement; (ii) the duration of hormone treatment; and (iii) the dose and type of sex steroids (Blázquez et al., 2001). The best time for hormonal exposure, termed as a critical period here, is the period where gonads reveal a high response to exogenous sex steroids.

The critical period for hormonal treatment can be identified through the application of hormone at different periods, as has been applied to different species such as European sea bass (Blázquez et al., 2001); rainbow trout (Cousin-Gerber et al.,

1989; Kato et al., 2001); honmoroko, *Gnathopogon caurulescens* (Fujioka, 1993); and red sea bream, *Pagrus mayor* (Kato et al., 2001) or by identifying early gonadal development through microscopic examination, as used in this experiment.

The present study demonstrated that female silver perch can easily be converted into males. The dietary supplementation of MT from 31 to 60 dph showed high sensitivity to exogenous androgen and resulted in complete masculinization at relatively low dosages and over a short period of time. The sex undifferentiated period for silver perch that is identified before two months of age (Moiseeva, 2001) showed its sensitivity to hormonal treatment. This period is shorter than another hermaphrodite fish, European sea bass, whose equivalent period lasts 11 months, during which the gonads remain sexually undifferentiated (Blázquez et al., 2001; Navarro-Martín et al., 2009; Saillant et al., 2003). However, the silver perch is still comparable to the other short sexually undifferentiated periods of fish such as the 30 mm fork length and 45 days onwards for Atlantic halibut, *Hippoglossus hippoglossus* (Hendry et al., 2002); the 19 mm total length (TL) for Atlantic cod, *Gadus morhua* (Chiasson et al., 2009; Haugen et al., 2011), and approximately 15 mm and 30 days for golden rabbitfish, *Siganus guttatus* (Komatsu et al., 2006).

The dietary dosage of 9 and 18 mg MT/kg diet resulted in a high percentage of males (Fig. 2) which is comparable to results from experiments involving rainbow trout and European sea bass, although a relatively higher dosage was applied to Nile tilapia, blue tilapia, and redfin perch (Table 2). In contrast, a relatively low dosage has also been reported to completely masculinize black tilapia, *Tilapia mosambica* and rainbow trout (Table 2). While a small MT dosage can result in incomplete masculinization, an overdose can, potentially, lead to infertility and even inconsistent feminization resulting aromatization of androgens to oestrogens (Goudie et al., 1983). Since the 9 and the18 mg MT/kg diet resulted in the same percentage of males, it is therefore using 9 mg MT/kg in silver perch diet is recommended.

The duration of hormonal exposure for 30 days in this study started when the fish were ready to accept the formulated diet, at 31 dph, to the time of sex differentiation, at 60 dph. The result showed that the duration is sufficient to completely masculinize silver perch juveniles. The same length of MT exposure has been reported by Rougeot et al. (2002) for redfin perch, but at higher MT concentration (40 mg/kg diet). Nearly the same duration is also reported for blue tilapia, which were fed for 28 days, at a concentration of 30 mg MT/kg diet (Tayamen and Shelton, 1978). The period before sex differentiation, identified histologically by Moiseeva (2001) coincides with the critical period for MT treatment in silver perch.

The average gonadal weight, length and GSI of fish did not show any significant ($P > 0.05$) differences due to any dietary supplementation of MT. Morphological differences in sex-reversed gonads have been reported in European sea bass (Blázquez et al., 2001) where androgen treatment encouraged a robust obstruction of the gonadal development, producing in reduced testes sizes with a great content of fibrous-connective tissues. However, in our research, there were no histological abnormalities in the male gonad of control fish or MT-fed fish at 225 dph, suggesting that sex-reversed genotypic males (neo-females) may be able to produce viable sperms. Similarly, male-functional blue tilapia (Guerrero, 1975), yellow catfish, *Pelteobagrus fulvidraco* (Liu et al., 2013), and rainbow trout (Cousin-Gerber et al., 1989) reversed from genotypic females, were able to produce viable sperms.

Histological analysis indicated no degraded female cells in the male gonads of MT-fed fish, indicating that MT induces the direct development of males, which is different from the normal sequence of sexual change from male to female (protandrous hermaphrodite) resulting in visibly degraded male cells (Moiseeva, 2001). The female gonads of control groups showed their normal development where different stages of the oocyte were visualized with the sporadic presence of degraded male cells. This indicates that natural females have to go through male stages prior to transforming into the female. When MT was applied to the fish larvae before the onset of sex differentiation, genetically female fish were sexually reversed to male before their female gonads were developed.

In the present study, the potential of different doses of MT to promote growth rate was also investigated. Monosex silver perch did not show significant difference in their TL after being fed MT. However, feeding MT appears to have a higher anabolic effect given that the weight gained by the fish treated with MT was higher than the control groups, after 195 and 225 dph. Hence, the dietary MT not only influences the sex ratio in favour of males but can also promote growth, as in Nile tilapia (El-Greisy and El-Gamal, 2012; Little et al., 2003). Dietary MT has also proven to be an effective growth promoter in: common carp (Nagy et al., 1981); coho salmon (Shelbourn et al., 1992); European sea bass (Navarro-Martín et al., 2009); rainbow trout (Cousin-Gerber et al., 1989); red sea bream, *Chrysophrys major* (Woo et al., 1993); and golden barb, *P. gelius* (Montajami, 2012).

The reasons for the enhanced growth performance in this study might have been a result of the direct feeding of MT which led to increased appetite, resulting in higher SGR. Other studies in Nile tilapia, have also stated that the enhancement of food conversion efficiency on sex-reversed fry could have

**Table 2** Reported complete masculinization of different species treated at different doses of 17 α-methyltestosterone (MT).

| Species | MT doses (mg/kg diet) | References |
|---|---|---|
| Rainbow trout, *Salmo gairdneri* | 1–9 | Solar et al. (1984) |
| European sea bass, *Dicentrarchus labrax* | 10 | Blázquez et al. (1995) |
| Nile tilapia, *Oreochromis niloticus* | 60 | El-Greisy and El-Gamal (2012) |
| Blue tilapia, *Tilapia aurea* | 60 | Guerrero (1975) |
| Redfin perch, *Perca fluviatilis* | 40 | Rougeot et al. (2002) |
| Silver perch, *Bidyanus bidyanus* | 9–18 | This study |

attributed to the higher mean weights (El-Greisy and El-Gamal, 2012; Chakraborty et al., 2011).

While dietary MT induces increased appetite and food consumption in coho salmon (Fagerlund et al., 1979), it depressed the appetite and feeding rate in rainbow trout (Yamazaki, 1976). It is unclear whether dietary MT improved the growth directly or by MT's ability to masculinize the sex, as, for example, it is established that the male tilapia, under normal conditions, grows better than females. Hence, it can be stated that the response to a given steroid hormone is species specific (Kuwaye et al., 1993) which could promote higher growth rate, by means of increased food conversion, activate the formation of other androgenic anabolic hormones, and as the direct effect of MT on the gene expression in the muscle cells. In the present study, the increased feeding rate was observed in hormonal-fed fish, leading to higher food ingestion in silver perch.

Complete masculinization without abnormality can only be achieved if the right dose of hormones is used at the correct time. Gonadal sensitivity to dietary MT in 31–60 dph in fish larvae corresponds to body weight between 0.15 and 0.34 g and to total length between 21 and 27 mm. Since this critical period occurs just after early weaning onto artificial dry feeds (Phipps, 1999), sex androgen applied at the critical time can influence the gonadal development and overrule genetic sex determination (Devlin and Nagahama, 2002). The suggested critical period, derived from our results, is within the range of critical periods reported for other species. For example, the critical period in Nile tilapia occurs in the early larval stage, at 7–14 dph (Kwon et al., 2000); and in the European sea bass (Blázquez et al., 2001; Navarro-Martín et al., 2009) during the late juvenile stage, at 96–126 days post fertilization.

In conclusion, the application of MT at 9–18 mg MT/kg feed from 31 to 60 dph is effective in inducing masculinization in silver perch.

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