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

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KEYWORDS

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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

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