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EFFECT OF TESTOSTERONE PROPIONATE ON FRY OF SIAMESE FIGHTER (*Betta splenden*)

ALUM-UDENSI, O., [*]J. C. IKWUEMESI, P. IKECHUKWU AND B. C. ONUKAIRE
Department of Fisheries and Aquatic Resources Management, Michael Okpara University of Agriculture, Umudike, PMB 7267, Umuahia, Abia State, Nigeria
Corresponding author: jpikwuemesi@gmail.com, +234 806 560 9818, 0000-0002-9133-4780

ABSTRACT
The effects of different levels of testosterone propionate administered by inclusion in feed on growth, survival and sex-ratio of Betta splenden was studied. Four (4) treatments of experimental diets and a control with 3 replicates for each were set up, and the hormone was administered to the fry from day 2 for a period of 8 weeks. The results indicated that synthetic androgen testosterone propionate administered by inclusion in feed influenced the sex in B. splendens. Highest percentages of males (80% and 75%) were found at doses 0.75 and 1.0mg/L. At a dosage of 0.25mg/L, a high percentage of females was observed. Higher doses of the hormone showed lower survival compared to lower doses. The frequency of male fish in all the hormone-treated groups except the 100mg/kg group was significantly higher than that of the expected frequency of male fish in a normal population.

Keywords: Fish breeding; Ornamental fish; Sex reversal; Mono-sex populations

INTRODUCTION

The entire aquarium industry, including ornamental fish, aquarium accessories, aquarium fish feed, aquarium construction and public aquariums is estimated to be worth more than the US $20 billion annually with much contribution in the export trade by South East Asia (Pal, 2015; Yadav and Sharma, 2017; Alum-Udensi and Orji, 2019). Freshwater tropical aquarium fishes are of great' economic importance due to their attractive colour, shape, finnage, and behaviour (Mekdaeng, 2015; Alum-Udensi *et al.,* 2019a; Bardhan *et al,* 2021). Sex reversal in fish can occur as a result of disturbances in natural conditions or laboratory manipulations (Smith, 2005).

The maintenance and breeding of male populations have generated a great amount of interest in commercial ornamental fish production (Singh *et al.,* 2019). Different methods such as manual sorting, hybridization hormonal sex reversal and super male production has been applied to mono-sex population (Soumokil *et al*., 2020) These techniques have been applied to some fish species exhibiting sexual dimorphism with preference of male to females including guppy *(Poecilia reticulata)*, balloon molly *(P. latipinna*), sailfin molly, (*P. velifera), sunset platy* (*Xiphophorus variatus)*, dwarf gourami(*Colisa lalia)*, fighting fish (*Betta splendens)*, rosy barb(*Barbus conchonius)*, Convict cichlid (*Cichlasoma nigrofasciatum)*, and red Australian rainbow, (*Glossolepis incisus)* Muniasamy, *et al.,* (2019). The males of these species may command up to four times the price of females, encouraging the culture of all-male mono-sex populations with significant economic advantage (Mousavi-Sabet 2011).

Manual sorting was the first method to be used in the early days as it required little or no technology besides simple sorting, culling and discarding the unwanted sex. This method is wasteful, inefficient (Hafeez-ur-Rehman *et al.,* 2008), stressful to fish, results in relatively high mortality (Dunham, 2004), requires experienced labour and the fish must be grown to the size of sexual dimorphism before commencement of manual sorting. Hybridization has also been used to produce mono-sex populations (Bartley *et al*., 2001; Bardhan *et al.,* 2021). Hybridization however, has some setbacks, including skewed sex ratios, production of infertile F1 generation (sterile offspring) and the need to maintain two separate pure brood lines (Mubarik *et al.,* 2011; Bartley *et al*., 2001)

Hormonal sex reversal involves use of hormones to effect sex reversal in fish. The treatment of sexually undifferentiated fry by administration of hormones has been shown to work well in a wide range of species (Pandian and Kirankumar, 2003; Bardhan *et al,* 2021). Badura and Friedman (1998) reported the transformation of the anatomical features/characteristics of females' fish injected with testosterone to males expressed by changes in fin length, body coloration, and gonadal morphology. 17α-methyltestosterone is the most preferred and widely used hormone for induction of masculinization in fish like *Betta splendens* (Amiri-Moghaddam *et al.,* 2010; Balasubraman, 2010).

Testosterone Propionate has a faster rate of release than other esterified testosterones, and it is also a locally available steroid. Different methods have been used to administer steroids, including immersion (Pandian and Kirankumar, 2003, Mekdaeng, 2015) however, oral administration of feed incorporated with methyltestosterone is the most effective and practical method for the production of all-male populations. Oral administration of the synthetic androgen 17α-methyltestosterone (MT) has been effective for all male populations in carp (Damstra *et al.,* 2002; Mubarik *et al.,* 2011). Bharadwaj and Sharma (2000) studied the effect of methyltestosterone (tablets) in sterilization and masculinization of common carp, *C. carpio var. communis (L).* Wassermann and Afonso (2003) investigated the efficacy of three androgens (17α-methyltestosterone, 17α-methyl dihydrotestosterone and

17α- ethynyltestosterone) through immersion treatment on the sex ratio of Nile tilapia *(0. niloticus)* fry. In undifferentiated fry, 50mg Trenbolone Acetate/ kg feed or immersion in TBA induced 100% male populations in both channel catfish, *Ictalurus Punctatus*, and blue tilapia, *Oreochromis aurea* (Fitzpatrick *et al.* 2000). Amiri-Moghaddam *et al.,* (2010) used 17-α-methyltestosterone to achieve male secondary sexual characteristics in the adult female green swordtail (Xiphophorus hellerii). Numerous attempts have been made to optimize the methods of hormonal sex reversal by varying parameters such as hormone dose, treatment start time, duration of treatment and stocking density (Kirankumar and Pandian, 2002; Mubarik *et al.,* 2011; Bardhan *et al,* 2021).

The Siamese fighting fish (*Betta splendens*) is a popular freshwater ornamental species that has been selectively bred to display a vibrant array of colour and tail types over the years (Alum-Udensi *et al.,* 2019a). The fish is sexually dimorphic, with males being more brightly colored with extravagant finnage than females. We investigated the use of testosterone propionate in hormonal sex reversal by incorporating it in the diet of *B. splendens.*

MATERIALS AND METHODS

Experimental design.

This study was carried out in the Department of Fisheries and Aquatic Resources Management, Michael Okpara University of Aquaculture, Umudike, Umuahia, Abia State, for a period of 56 days (8 weeks). Three pairs of broodstock *Siamese fighter* were procured from Nature Nurture - an ornamental fish breeder in Abuja, Nigeria and transported to Umudike. Spawning was done to obtain fry. A total of 225 fry of *B. splendens* at two (2) days old were carefully collected in two transparent plastic tanks before separation into the various treatments and replicates. Transparent plastic tanks of (40 x 30x 40) cm each were set up for five (5) treatments representing experimental diets with 3 replicates for each. The tanks were filled with water to about 10cm, and twelve (15) *B. splendens* fry were randomly transferred into each experimental tank for the feeding trials.

Hormone preparation and administration

The stock solution of testosterone propionate was prepared in absolute ethanol at a concentration of 1mg testosterone propionate/ml ethanol. The treatment was diluted appropriately to the final steroid concentration for each treatment diet and sprayed over the powdered fry food and left open at room temperature for 24 hours for the ethanol to evaporate, leaving behind hormone-incorporated feed.

Feeding regime and water quality

Commercial fry feed containing 65% crude protein was procured, and the hormone was incorporated at four different rates: 25(T1), 50(T2), 75(T3), and 100(T4) $mg.kg^{-1}$. 0(T5) served as the control diet without hormones to the food. The fry were fed with fry food containing various levels of testosterone propionate. Partial water change in the experimental tanks was carefully done once a week; however, 1 litre of fresh water was added to each tank daily after feeding. Water parameters (temperature, pH, dissolved oxygen and ammonia using Hanna model HI83303, made in Italy) were monitored weekly.

Data collection and analysis

Fry weight was determined using a sensitive weighing balance (PL303 Mettler Toledo from China) to the nearest 0.1g. Mortality was counted and recorded; the Sex of experimental fish was determined at about 2.5 cm body length by the external examination of the dorsal, anal, and caudal fins following Kirankumar and Pandian, (2002). Growth, survival and percentage weight gain were computed and analyzed using analysis of variance (ANOVA), while means were compared using turkey's HSD

RESULTS

The percentage survival of *B. splendens* after inclusion of the hormone in feed during the first 8weeks for each Treatment is given in Figure 1. The percentage survival rate was also 100% for all the treatments (0.25 $mg.kg^{-1}$, 50 $mg.kg^{-1}$, 75 $mg.kg^{-1}$, and 100 $mg.kg^{-1}$) in week 1. After 8weeks, the survival rates went down to 80%, 66.7%, and 53.3% in 0.50, 0.75, 1.00mg respectively. This change in the percentage survival was also observed in the control suggesting non hormone influence. The effect of testosterone propionate on sex ratio of *B. splendens* was also studied and the results were presented in Table 2. The sex ratio in the treatments 0.25mg/L, 0.50mg/L, 0.75mg/L and 1.00mg/L were 1:0.71, 1:0.5, 1:0.25 and 1:0.22 respectively. The water quality was maintained at optimal range, pH (7-7.2), Temperature (25-27^{0}C) and Total ammonia (0.05-0.07mg/l)

Table 1 presents the effect of testosterone propionate on the growth and survival of *B. splendens* was also studied for the four doses viz 0.25mg, 0.50mg, 0.75mg and 1.0mg and zero for the control groups. The first treatment 0.25mg had a mean weight of 0.032±0.041, 0.131±0.04, and 0.189±0.021 after 2weeks, 6weeks and 8weeks respectively. The second treatment had a mean weight of 0.038± 0.07 and 0.204±0.041 after 2 weeks and 8 weeks of monitoring. In week 2, treatment 3(0.75mg) showed a mean least weight of 0.031±0.014 and increased to mean weight of 0.157±0.082 and 0.192±0.014 after week 6 and 8weeks, respectively. However, the treatment 4(1.0g) had a mean weight of 0.037±0.021 and 0.207±0.04 after 2 weeks and 8weeks, respectively while the control was after 2 week and after 8weeks. There was no significant different at week 2 in all the treatments. At week 8, T2 and T4 are significantly higher than other treatments at $p<0.05$. Highest percentages of males (80% and 75%) were found at dose 0.75 and 1.0mg/L (Table 2). At the dose of 0.25mg/L a high percentage of female were observed. There was no statistical difference between the control and T1 in the male and female ratio.

Table1: Effect of testosterone propionate on weight gain of *B. splendens*

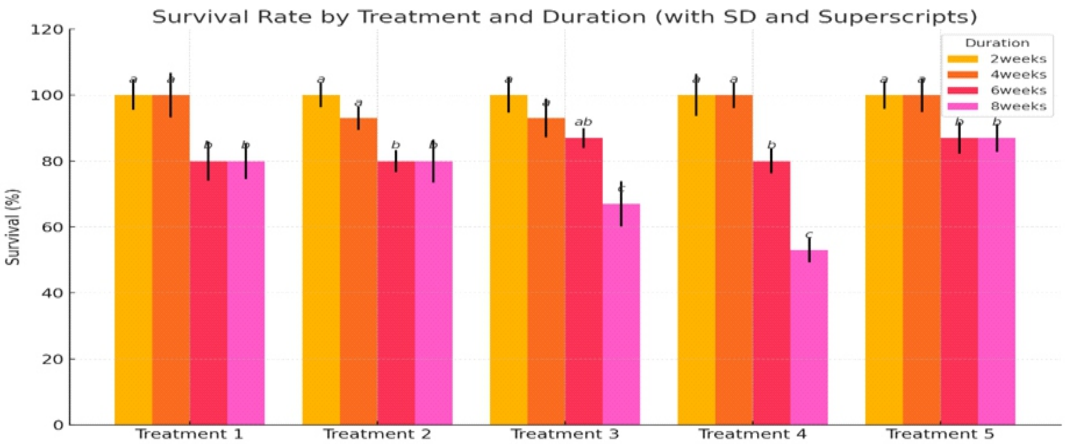
weeks	T1	T2	T3	T4	T5
2	0.032 ± 0.041^{a}	0.038 ± 0.07^{a}	0.031 ± 0.014^{a}	0.037 ± 0.021^{a}	0.034 ± 0.02^{a}
4	0.084 ± 0.01^{b}	0.076 ± 0.02^{b}	0.087 ± 0.01^{b}	0.091 ± 0.001^{b}	0.088 ± 0.02^{b}
6	0.131 ± 0.04^{a}	0.154 ± 0.002^{b}	0.157 ± 0.082^{b}	0.177 ± 0.03^{c}	0.092 ± 0.02^{b}
8	0.189 ± 0.021^{a}	0.204 ± 0.041^{b}	0.192 ± 0.014^{a}	0.207 ± 0.04^{b}	0.188 ± 0.07^{a}

Treatments with the same superscript along the row are not significantly different @p<0.05

Table 2: Effect of testosterone propionate on the sex ratio of *B. splendens.*

Treatment	No of sexed fish	Males	Females	Sex ratio
T1	36	21 ± 2^{a} (58.3%)	15 ± 1^{a} (41.7%)	1:0.73
T2	36	24 ± 1^{b} (66.7%)	12 ± 1^{c} (33.3%)	1:0.5
T3	30	24 ± 2^{c} (80%)	6 ± 1^{b} (20.0%)	1:0.25
T4	24	18 ± 2^{c} (75%)	6 ± 1^{b} (20.0%)	1:0.33
Control	39	21 ± 2^{a} (53.8%)	18 ± 1^{a} (46.2%)	1:0.86

Treatments with the same superscript along the column are not significantly different @p<0.05



*Bars with same alphabet are not statistically different at p>0.05

Figure 1: Survival of *Betta splenden* treated with testosterone propionate

DISCUSSION

The present study demonstrates that dietary administration of testosterone propionate can significantly influence sex differentiation in *Betta splendens*, with specific doses yielding a pronounced masculinizing effect. The trend observed aligns with previous studies on related species, suggesting that the application of exogenous androgens can be an effective tool in sex control strategies for ornamental fish culture.

The current findings corroborate the report by Al-Hakim *et al*. (2013), who observed superior weight gain and length in *Oreochromis niloticus* and *Cirrhinus mrigala* at 60 mg/kg doses of 17α-methyltestosterone (17α-MT). Similarly, Arul and Sindhu (2018) reported significantly improved growth performance and yield in *Clarias gariepinus* treated with 30–60 mg/kg feed of 17α-MT over a 28–35-day period. These results suggest a consistent dose-dependent effect of synthetic androgens on somatic growth across various freshwater fish species, including *B. splendens*. In our study, although weight differences between treatment and control groups were not statistically significant, the pattern of growth increase in hormone-treated groups may be indicative of a latent anabolic effect, which could be more pronounced under longer treatment durations or with multiple spawning cycles.

Environmental parameters such as pH, temperature, and ammonia remained within optimal ranges throughout the study period, thereby minimizing potential abiotic stressors (Boyd, 2015). This ensured that the observed differences in sex ratio and growth performance were attributable primarily to the hormonal treatments rather than environmental variation.

The control group exhibited a near 1:1 sex ratio (1:0.86 male to female), consistent with the expected natural distribution. However, treatment with 0.25 mg/L testosterone propionate did not significantly alter the sex ratio, suggesting that this dose may be below the threshold required to induce effective masculinization in *B. splendens*. This observation is supported by Pongthana and Tangthongpairoj (1999), who found that a lower dose (20 µg/L) of 17α-MT in *Clarias gariepinus* led to a higher proportion of females, indicating that insufficient hormonal stimulation may inadvertently favour feminization or fail to disrupt natural sex differentiation.

Of particular interest is the performance of the 0.75 mg/L treatment group, which yielded the highest proportion of males (80%) and the lowest proportion of females (20%). This suggests that 0.75 mg/L represents an optimal concentration for effective sex reversal in *B. splendens* under the conditions of this study. A slightly higher dose (1.00 mg/L) also resulted in a high male ratio (75%), although the number of successfully sexed fish was lower (n = 24), possibly due to increased mortality or adverse physiological responses to excess androgen exposure. These findings reflect a dose-dependent response and imply that beyond a certain threshold, increasing the hormonal dose may not yield proportionally better results and may instead compromise fish viability.

This dose-dependent masculinizing effect is consistent with the assertions of Yamazaki (2006), who highlighted the importance of hormone dose, method of administration, and the developmental stage of the fish in determining the success of androgen-induced sex reversal. The present results also align with observations by Demska-Zakes and Zakes (1997), who reported that although methyltestosterone (at 30–90 mg/kg) affected sex ratios in *Stizostedion lucioperca* (pikeperch), complete sex reversal was not achieved at any tested dose. This underscores the species-specific variability in responsiveness to hormonal sex reversal techniques.

Fish mortality observed across treatments, although not statistically significant, raises concerns about potential toxicity at higher hormone concentrations. It highlights the need for a balance between effective sex reversal and fish health. While sex ratio manipulation is desirable in ornamental fish production for phenotypic control and market preference, it must not compromise survival or overall performance.

CONCLUSION

Testosterone propionate influenced the sex of *B. splendens* males at the optimal dose but did not contribute to the enhancement of the growth of the hormone-treated fish. An all-male population could not be achieved in this study. There is a need for further studies to determine the optimal dose of hormone, duration and the best route of administration of the hormone, earlier developmental exposure, or combined hormone protocols to optimize outcomes in ornamental species.

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