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Efficacy of 17α-methyl testosterone on Growth and Survival of Fry of Swordtail, *Xiphophorus helleri*

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ABSTRACT

The experiment was carried out in the wet lab at the College of Fisheries, G.B.P.U.A. & T, Pantnagar, India, to compare the effectiveness of 17 α -methyl testosterone on Swordtail (*Xiphophorus helleri*) fry administered at various dose rates by oral feed administration. Fish fry was fed at 5% of body weight twice a day. In addition, the commercial feed was added with various doses of 17α-methyl testosterone *i.e.,* 30 mg/kg, 60 mg/kg, and 90 mg/kg, and one control aquarium tank (T_0) was installed for the comparative study with treatment aquarium tanks like H_1, H_2, and H_3, respectively. The effectiveness of 17α-methyl testosterone on the growth and survival of swordtail fish fry was observed after 90 days of this experiment. In 90 mg/kg (H_3) feed minimum growth (weight and length) was observed as compared to H_2, H_1 and T_0 respectively. Mortality of 16.67 % was also observed with 90 mg/kg (H_3) feed as compared to H_2, H_1 and T_0 respectively. The results showed that 17α-methyl testosterone has significantly negative effects on swordtail than the control experimental aquarium tank.

Key Words: Sword tail, 17α-methyl testosterone, Growth, Mortality.

INTRODUCTION

The habit of keeping ornamental fish in aquariums for display and entertainment dates back (Ayyappan *et al*, 2011). People kept vibrant tropical fish as a hobby in aquariums and garden ponds. Because of the vibrant body colours, unique morphologies, and eating habits, ornamental fish are living jewels. The practise of keeping fish in aquariums in India began during the British era and is still practiced (Ayyappan *et al*, 2011). Additionally, India is blessed with a favourable climate, an abundance of water resources, a diverse variety of flora and fauna, and a large pool of skilled labourers spread out across the entire nation.

Ornamental fish breeding and culture are expanding as a business in India. However, India only contributes a little amount to the ornamental fish market, and at the moment, the majority of the fish exported from India are wild-caught species. The decorative fish industry provides rural and urban communities with an excellent opportunity to increase their income and connects them to international trade. The vast majority of ornamental fish raised in tanks are from small-scale or backyard breeding operations.

Sword tails are small freshwater fish that are indigenous to Central and North America (*Xiphophorus helleri*). Swordtails are omnivores in natural water bodies and consume a variety of invertebrates, insects, plant material, and algae for food. As an ornamental fish, sword tail (*Xiphophorus helleri*) is valuable commercially (Moghaddam *et al*, 2010). This fish is a live-bearer. The value of phenotypic males—those with caudal fins that have sword extensions can be up to twice that of phenotypic females (i.e., fish lacking swords)

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(Yanong *et al*, 2006). Male swordtail fish have a more pronounced sword-like protrusion on the caudal fin and are smaller and thinner than females. Swordtails range in length from 6-7 cm for males to 7-9 cm for females. Temperatures between 23 to 28 °C and water hardness levels between 50 and 100 mg of $CaCO_3$ per liter (moderately soft water) are ideal for swordtail breeding (Ayyappan *et al*, 2011).

The effects of androgenic steroids on increasing growth have been extensively studied in salmonid fish, which includes both trout and salmon. Using the naturally occurring androgens testosterone and ll-ketotestosterone, Younis *et al*, (2023) discovered considerable weight gains in juvenile coho salmon (Oncorhynchus kisutch). Following the introduction of two synthetic androgens, dimethazine and norethandrolone resulted in increases in the growth rate of young rainbow trout (*Salmo gairdneri*) (Nynca *et al*, 2023). According to studies conducted on salmonids, the synthetic androgen 17α -methyltestosterone (MT) causes significant weight gain (Zhu *et al*, 2020; Liu *et al*, 2023). The study of androgens did not always result in weight gain. When the synthetic steroid stanozolol was tested by Suseno *et al* (2020) for its effects on channel catfish (*Ictalurus punctatus*), they discovered no discernible variations in growth between the various treatment doses. Using the synthetic androgen methandrostenolone, Zhu *et al* (2022) also gave channel catfish the treatment. They discovered no changes in total weight gain between the treated and untreated fish.

MATERIALS AND METHODS

Experimental site

The experiment was conducted in the Wet Laboratory of the College of Fisheries, Govind Ballabh Pant University of Agriculture and Technology in Pantnagar. It is located in the Tarai belt of the Himalayan foothills at a 243.3 metres height above mean sea level (MSL) and has the coordinates 290ºN latitude, 79.30ºE longitudes. Pantnagar's humid subtropical climate is characterised by a dry, summer and a relatively chilly winter, with the mist often appearing at the last of December and through the end of February. The study was conducted for a period of 90d i.e. February to May. This experiment aims, were to determine the effectiveness of 17-Methyltestosterone (MT) on swordtail fish growth when applied to fish fry. For the experiment, 120 fries in total were taken. There were 12 groups of 10 fries each. Each aquarium contained 10 swordtail (*Xiphophorus helleri*) fries and triplicates of each treatment were made.

Fish fry source and experimental aquariums

Around 200 fish fry (*Xiphophorus helleri*) were collected from College of Fisheries available brooders of swordtail fish, and others were purchased from nearby market. On the other hand, aquariums made up of glass for our experiment since ornamental fishes fall within that category.

Experimental design

Twelve fabricated aquariums measuring 4.0×1×1 foot was installed for the duration of this 90-day experiment, with an approximate water depth of 0.65 foot (approx. Capacity of 80 litres). The experimental aquarium was initially cleaned and sterilised with lime and $KMnO_4$, then filled with tap water and continuously aerated using aerators, respectively. Throughout the course of the study, siphoning with a 25% water exchange were used to clean the aquarium and remove residual feed and excreta. The aquarium was continuously aerated and refilled with fresh tap water from a bore well. Water heaters were used to keep water at a consistent temperature during the winter. According to the experimental plan, the control tanks were designated "T_0A, T_0B, T_0C" whereas the treatment tanks were designated "H_1A, H_1B, H_1C" for 30 mg of hormone per kg of feed, "H_2A, H_2B, H_2C" for 60 mg of hormone per kg of feed, and "H_3A, H_3B, H_3C" for 90 mg of hormone per kg of feed.

17-α methyl testosterone stock solution preparation and hormone infused feed

The hormone was diluted in 96% ethanol to a concentration of 1 mg/ml to create a stock solution of 17- α methyl testosterone, which was then stored at 4 °C in the refrigerator. Dried pelleted aquarium feed (Brand- Pacific green) was extracted and pulverised into powdered form to prepare hormonal mixed feed for fry. On three disposable plates, 100 g of feed was taken. Following the alcohol evaporation process, the stock solution of MT was then added to the feed stored in the plates in various amounts to obtain the hormone concentration of 30, 60, and 90 mg/kg of feed (Guerrero *et al,* 1975). As a control, one plate of feed was made without hormone. Different doses of hormone-mixed feed were kept apart in an airtight zip plastic bag and kept in the refrigerator. Feed was utilised after it had been brought to room temperature. To avoid confusion, feeding was done with specialised plastic made spoons for various hormone-mixed feed concentrations.

Feeding schedule

Fish were given the hormone-mixed meal twice daily at a rate of 5% of the fry body weight. Following 15 d, sampling was carried out to determine the amount of feed to use in accordance with the fish recorded weights.

Growth measurement

Growth performance of fry was determined for a period of 90 d with an interval of 15 d sampling (Initial, 15^{th}, 30^{th}, 45^{th}, 60^{th}, 75^{th} & 90^{th} day). Periodic sampling was done fortnightly for monitoring the growth performance. Growth rate was recorded by determining the mean length (cm) and mean weight (g) of fry of different treatment groups. Body weight was taken by electronic balance and length was taken by centimetre scales. Special care was taken while handling those tiny fries.

Survival percentage

The survival rate of the fry was determined as number of fishes stocked divided by number of fishes survived.

Statistical analysis

The rate of growth performance and survival rate of fry were statistically analysed (ANOVA) by using the Microsoft Excel (2013).

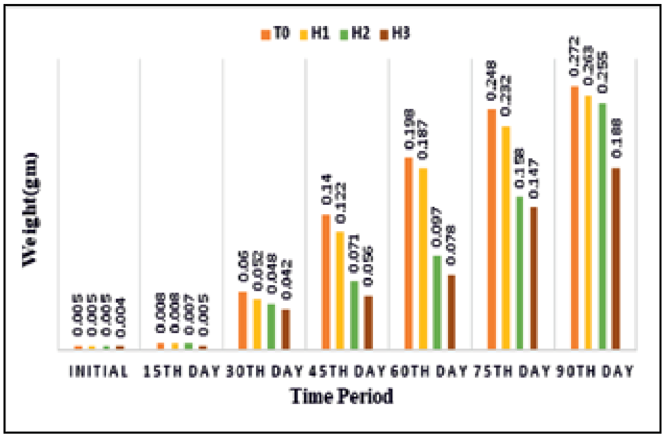
RESULTS AND DISCUSSION

Hormones and their effect on growth

For the purpose of masculinization and growth in a number of fish species, the androgen 17 α-methyltestosterone (MT), an anabolic steroid, is frequently utilized (Moghaddam *et al*, 2010). Widespread use of 17 α -Methyltestosterone today due to its ease of absorption, lack of accumulation in fish bodies, and ease of excretion (Al-Ansari *et al*, 2013).

The effect of residual hormone on growth performance of fry was determined by comparing the average length and weight of the fry between treatment groups and control group at different time intervals. The average length of fry was found day wise significantly ($P<0.05$) decreasing in all treatment groups (H_1, H_2 and H_3) except the control group (T_0) (Fig.1). When the length of treatment groups compared among themselves and with control group found significantly different ($P<0.05$). Similar observation was noted for the average weight of the fry. The weight of treatment groups (H_1, H_2 and H_3) was found significantly different ($P<0.05$) (Fig.2).

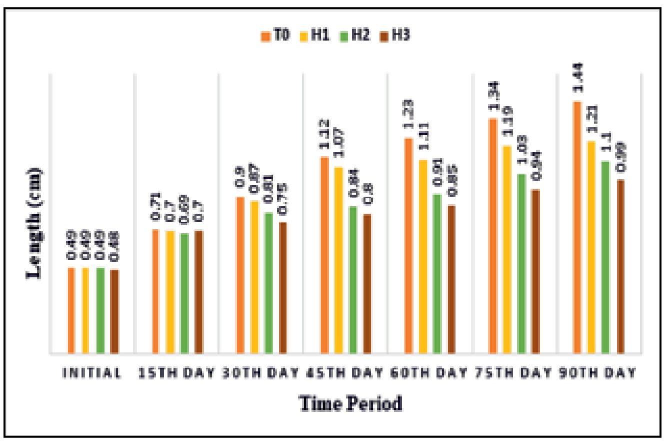
Feeding MT suppressed growth rate and caused morphological anomalies in fish at different dietary concentrations. Control fish had the highest weight gain, followed by the fish fed 30 mg of MT/kg of diet ($P< 0.05$) (Fig.2). The fish fed 90 mg of MT/kg of diet had less gain than those fed 30 mg of MT/kg ($P< 0.05$). During the study period mortality of the fry was observed in control group T_0 and treatment groups H_1, H_2 and H_3 respectively (Fig.3).



T_0: Control

H_1, H_2 and H_3: Feed with different hormone concentration

Fig. 1: Mean length (cm) of sword tail fry in different tanks with hormone



T_0: Control

H_1, H_2 and H_3: Feed with different hormone concentration

Fig. 2: Mean weight (gm) of sword tail fry in different tanks with hormone feed

MT was fed to fish at dosages close to the lowest concentration employed in previous research, particularly with cold water fishes (Othman *et al*, 2022; Farias *et al*, 2023), they suggested that MT generally boosted growth. The amount of food given with hormone or how long it was given to the fish could be contributing factors in the current study's decreased growth rate (Liu *et al*, 2023), and certain salmonids. High dietary doses of MT inhibited the growth of carp (Marjani *et al*, 2009).

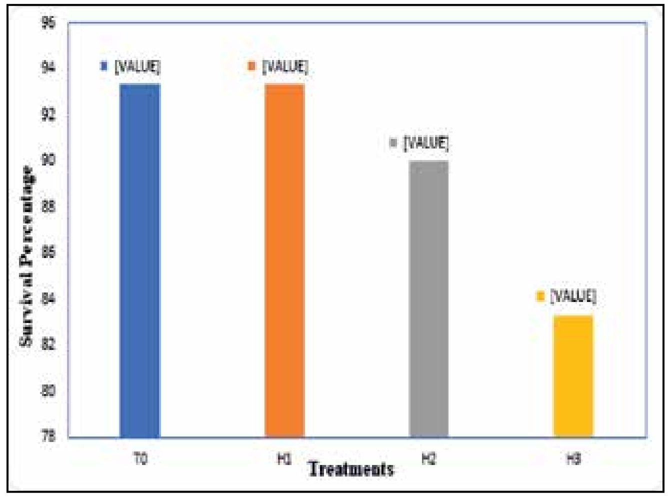


Fig. 3: Survival percentage of fry reared in different tanks with hormonal feed

A negative correlation between hormone dose and growth response was also found by Manosroi *et al* (2004) in fish given MT. The present study indicated lower growth suppression as less doses of hormone were fed. There were negative effects of hormone on survival due to increasing of hormone concentration recorded by (Manosroi *et al,* 2004; Gullu *et al,* 2005; Somrudee and Powapol (2008). The findings of the present study were in agreement with Ismihan *et al* (2006) that survival percentage reduces when more amount of hormone is added to feed.

CONCLUSION

These findings demonstrate that feeding swordtail MT at higher levels of food was ineffective. The dose level employed or the length of the feeding period may be responsible for the unfavourable reactions seen. Because MT is physiologically transformed into substances that are not anabolic, swordtail may react to MT differently than some of the other species investigated. It might be necessary to do more research using other feeding regimens for swordtail or lower dietary levels of MT.

REFERENCES

Al-Ansari A M, Atkinson S K, Doyle J R, Trudeau V L and Blais J M (2013). Dynamics of uptake and elimination of 17α-ethinylestradiol in male goldfish (*Carassius auratus*). *Aqua toxic* **132:** 134-140.

Amiri-Moghaddam J, Maniei F, Mahboobi S N and Asadollah S (2010). Use of 17α-methyltestosterone for production of male secondary sexual characteristics in the adult female green swordtail (*Xiphophorus hellerii*). *Intl J Bioflux Society* **3**(1): 1-8.

Ayyappan S, Jena J K, Gopalakrishnan A and Pandey A K (2011). *Handbook of fisheries and aquaculture*. Indian Council of Agricultural Research. Directorate of Information and Publications on Agriculture. 185- 93.

Farias R D S, Oliveira K R D S, Souza M E D, Ferreira D A, Silva A A D N, Silva Júnior V A D, Dunham R and Coimbra M M (2023). Effect of dosage of orally administered 17α-methyltestosterone on sex reversion of the yellowtail tetra *Astyanax lacustris* (Lütken, 1875). *Anim Reprod* **20**: 20220080.

Guerrero R D and Guerrero L A (1998). *Feasibility of commercial production of sex-reversed Nile tilapia fingerlings in the Philippines*. In: The Second International Symposium on Tilapia in Aquaculture. ICLARM Conference Proceedings 15: 183-86.

Gullu K, Guzel S and Yusuf G (2005). Effect of Estradiol Valerate applied by immersion and oral administration on growth and sex reversal of Rainbow trout, *Oncorhynchus mykiss. Biotechnol* **4**(3): 202-205.

Ismihan K, Orhan A K and Sedak K (2006). Effect of different levels of 17α-Methyltestosterone on growth and survival of angelfish *(Pterophyllum scalare,* Liccchtenstein, 1923) fry. *J Anim Vet Adv* **5**(3): 244-48.

Liu S, Chen Y, Li T, Qiao L, Yang Q, Rong W, Liu Q, Wang W, Song J, Wang X and Liu Y (2023). Effects of 17α-Methyltestosterone on the Transcriptome and Sex Hormones in the Brain of *Gobiocypris rarus*. *Int J Mol Sci* **24**(4): 3571.

Liu S, Xu P, Liu X, Guo D, Chen X, Bi S, Lai H, Wang G, Zhao X, Su Y and Yi H (2021). Production of neo-male mandarin fish *Siniperca chuatsi* by masculinization with orally administered 17α-methyltestosterone. *Aquaculture* **530**: 735904.

Manosroi J, Petchjul K and Manosroi A (2004). Effect of fluoxymesterone fish feed granule on sex reversal of the hybrid, Thai Red tilapia (*Oreochromis niloticus* Linn. X *Oreochromis mossambicus* Linn.). *Asian Fish Sci* **17**: 323-31.

Marjani M, Jamili S, Mostafavi PG, Ramin M and Mashinchian A (2009). Influence of 17-alpha methyl testosterone on masculinization and growth in tilapia (*Oreochromis mossambicus*). *J Fish Aquat Sci* **4**(1): 71-74.

Nynca J, Malinowska A, Świderska B, Wiśniewska J, Dobosz S and Ciereszko A (2023). Triploidization of rainbow trout affects proteins related to ovary development and reproductive activity. *Aquaculture* **565**: 739145.

Ostrowski A C and Garling Jr D L (1988). Influences of anabolic hormone treatment and dietary protein: energy ratio on condition and muscle deposition of rainbow trout. *Prog Fish C* **50**(3): 136-40.

Othman R, Ron X J, Yao H, O'Bryant P, Rapp D, Pei JC, Wu H J and Wang HP (2022). The effect of methyltestosterone (MT) on sex differentiation and growth in juvenile yellow perch (*Perca flavescens*). *Fish Physiol Biochem* **48**(1): 61-171.

Somrudee S and Pawapol K (2008). Masculinisation of flowerhorn by Immersion in androgens. *Silpkorn U Sciences Tech* **2**(2): 26-32.

Suseno D N, Luqman E M, Lamid M, Mukti A T and Suprayudi M A (2020). Residual impact of 17α-methyltestosterone and histopathological changes in sex-reversed Nile tilapia (*Oreochromis niloticus*). *Asian Pacific J Reprod* **9**(1): 37.

Yanong R, Hill J, Daniels C J and Watson C A (2006). Efficacy of 17α- Methyltestosterone for Expression of male Secondary Sexual Characteristics in the Swordtail. *N Am J Aquac* **68**: 224-29.

Younis M E, Jaber F A, Majrashi K A, Ghoneim H A, Shukry M, Shafi M E, Albaqami N M, Abd El-Hack M E and Ghanima M M (2023). Impacts of synthetic androgen and estrogenic antagonist administration on growth performance, sex steroids hormones, and immune markers of male and female broilers. *Poult Sci* **102**(1): 102244.

Zhu Q, Han C, Liu S, Ouyang H, Liu D, Zhang Z, Huang J, Han L, Li S, Li G and Lin H (2022). Development and gene expression analysis of gonad during 17α-methyltestosterone-induced sex reversal in mandarin fish (*Siniperca chuatsi*). *Aquac Rep* **23**: 101049.

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